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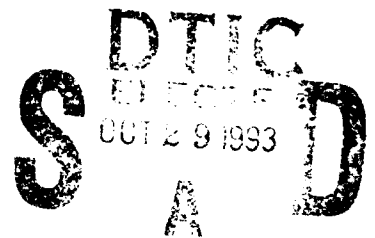
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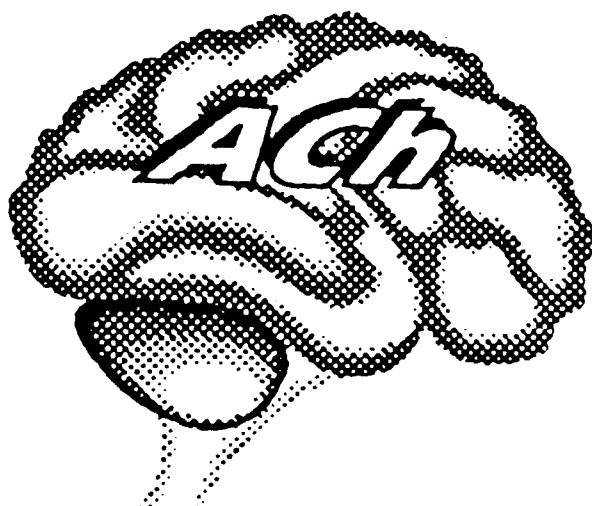
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# CHOLINERGIC NEUROTRANSMISSION: FUNCTION AND DYSFUNCTION



8th International Cholinergic Symposium

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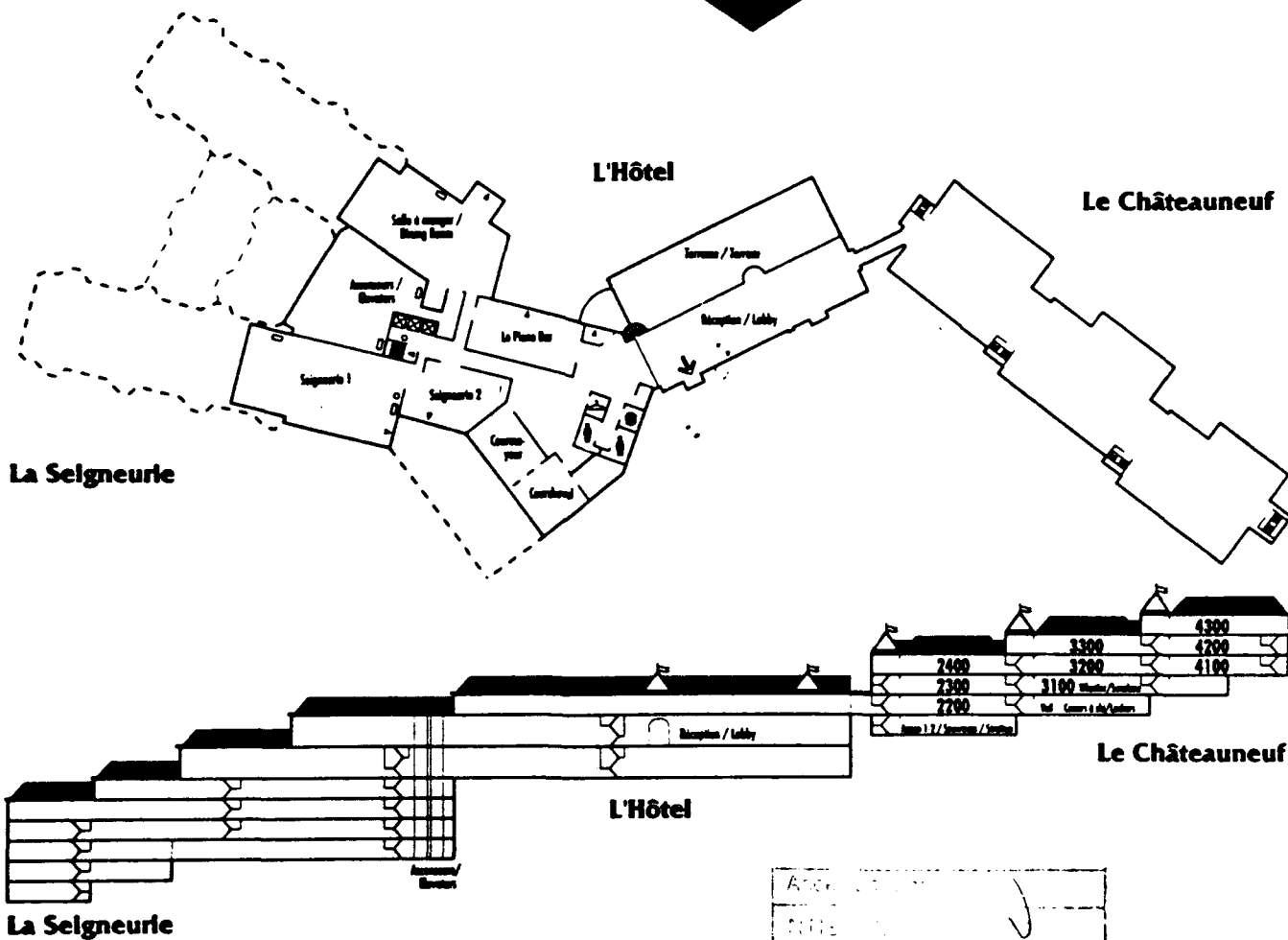
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# Complexe hôtelier LE CHANTECLER



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### La Seigneurie

Salle de réunion Meeting Rooms	Niveau Level	Chambres Rooms	Niveau Level
Chamonix 1 & 2	B	5700	B
Courchevel	A	5600	C
Courmayeur	A	5500	D
Davos	C	5400	E
Garmish	C	5300	F
Méribel	C	5200	G
La Clusaz	F	5100	H
Les Arcs	D		
Le Piano Bar	A	Salle à manger / Dining Room	A
Seigneurie 1 & 2	A	Salon audio-visuel /	F
Solden	E	Audio Visual Room	
St-Anton	C	Casiers à Ski / Ski Lockers	D
Tignes 1 & 2	B		
Val Gardena	C		
Verbier	C		
Villars	E		
Wengen	F		
Zermatt	D		

### L'Hôtel

Étage inférieur	Lower level
Bar Cocorico	Beauty salon
Billetterie de ski	Cafeteria
Boutique de ski	Cocorico Bar
Cafeteria	Hairdresser
Centre Sportif	Lift tickets
Écode de ski	Nautilus
Nautilus	Pool
Piscine	Sauna
Salon de coiffure	Ski boutique
Salon d'esthétique	Ski School
Sauna	Sports center
Théâtre	Theater
2ième étage	2nd floor
Chambres: 1201 - 1231	Rooms: 1201 - 1231

### Le Châteauneuf

Salles de réunion Meeting Rooms	Chambres Rooms
Aspen 1 & 2	2201 - 2212
Snowmass	2301 - 2310
Stratton	2401 - 2408
Sunshine	3101 - 3107
Vail	3201 - 3211
Whistler	3301 - 3310
Casier à ski/	4101 - 4111
Ski Lockers	4201 - 4211
	4301 - 4310

## MEETING ORGANIZATION

### Local Organizing Committee

A. Claudio Cuello (Convener)  
Paul Clarke  
Brian Collier  
Robert Dykes  
Serge Gauthier  
Kresimir Krnjević  
Remi Quirion

### Symposium Co-ordinating Committee

Paul Clarke (Chairman)  
Dusica Maysinger  
Maryka Quik  
Sharon Welner

## FINANCIAL CONTRIBUTIONS

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# *C O N T E N T S*

<b>Meeting Schedule</b> .....	<b>1</b>
<b>Scientific Program</b> .....	<b>3</b>
<b>Instructions for Symposium Speakers</b> .....	<b>9</b>
<b>Instructions for Poster Presenters</b> .....	<b>9</b>
<b>Symposium and Poster Abstracts</b> .....	<b>10</b>
<b>First Author Index</b> .....	<b>87</b>
<b>Meeting Participants</b> .....	<b>90</b>



# ***MEETING SCHEDULE***

## **Sunday July 26, 1992**

- 2:00 - 7:00            Registration
- 7:00 - 9:00            Opening Reception in Seigneurie 1

## **Monday July 27, 1992**

- 8:15 - 8:30            Introductory Remarks
- 8:30 - 10:00           Session 1: Organization of central cholinergic systems
- 10:00 - 11:00           Posters/Coffee Break
- 11:00 - 12:30           Session 2: CNS distribution of receptors
- 
- 4:00 - 5:30            Session 3: Molecular aspects of receptors
- 5:30 - 6:00            Posters/Coffee Break
- 6:00 - 7:30            Session 4: Molecular biology of cholinesterases

## **Tuesday July 28, 1992**

- 8:30 - 10:30           Session 5: Synthesis and storage of ACh
- 10:30 - 11:00           Posters/Coffee Break
- 11:00 - 12:30           Session 6: ACh turnover and release
- 
- 4:00 - 5:30            Session 7: Molecular aspects of ACh release
- 5:30 - 6:00            Posters/Coffee Break
- 6:00 - 7:30            Session 8: Trophic interactions
- 9.30 - 10.30           Local Organizing Committee and Advisory Board - Business meeting

**Wednesday July 29, 1992**

8:30 - 10:00	Session 9: Electrophysiological aspects of cholinergic mechanisms
10:00 - 11:00	Posters/Coffee Break
11:00 - 12:30	Session 10: Second messengers
4:00 - 5:30	Session 11: Modulation of information
5:30 - 6:00	Posters/Coffee Break
6:00 - 7:30	Session 12: Behavioural aspects of cholinergic transmission (Forum for discussion)
8:00	Banquet

**Thursday July 30, 1992**

8:00 - 9:30	Session 13: Cholinergic involvement in sleep and arousal
9:30 - 9:45	Coffee Break
9:45 - 12:15	Session 14: Clinical aspects of cholinergic pharmacology
12:15 - 12:45	Closing lecture
12:45 - 1:00	Farewell remarks
1:00	Closing Luncheon

**All sessions will be held in Seigneurie 1 except for session 12, which will be held in Chamonix 1 & 2**

# ***SCIENTIFIC PROGRAM***

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## **INTRODUCTORY REMARKS - A. Claudio Cuello**

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### **Session 1: ORGANIZATION OF CENTRAL CHOLINERGIC SYSTEMS**

**Chair:** Barbara Jones (McGill University, Montreal, Canada)

**Speaker:** Larry Butcher (UCLA, Los Angeles, California, USA)  
Cholinergic neurons in the rat central nervous system demonstrated  
by *in situ* hybridization of choline acetyltransferase mRNA

Donald Price (Johns Hopkins University, Baltimore, USA)  
Cholinergic systems: Abnormalities in neurological disease and  
prospects for therapy

**Discussant:** Bruce Wainer (University of Chicago, Chicago, USA)

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**Session 2: CNS DISTRIBUTION OF RECEPTORS**

**Chair:** Sten-Magnus Aquilonius (Uppsala Univ., Uppsala, Sweden)

**Speakers:** Paul Clarke (McGill University, Montreal, Canada)  
CNS nicotinic receptors: localization and relation  
to cholinergic transmission

Remi Quirion (McGill University, Montreal, Canada)  
Comparative distribution of multiple muscarinic receptor  
sub-types in the mammalian brain

**Discussant:** José Palacios (Laboratorios Almirall, Barcelona, Spain)

---

**Session 3: MOLECULAR ASPECTS OF RECEPTORS**

**Chair:** Herbert Ladinsky (Inst. Angeli SPA, Milan, Italy)

**Speakers:** James Patrick (Baylor Coll. of Med., Houston, Texas, USA)  
Functional diversity of neuronal nicotinic  
acetylcholine receptors

Mark Brann (University of Vermont, Burlington, USA)  
Structure/function relationships of muscarinic  
acetylcholine receptors

**Discussant:** Sir Arnold Burgen (Univ. of Cambridge, Cambridge, England)

---

**Session 4: MOLECULAR BIOLOGY OF CHOLINESTERASES**

**Chair:** Edith Heilbronn (University of Stockholm, Stockholm, Sweden)

**Speakers:** Jean Massoulié (Ecole Normale Supérieure, Paris, France)  
Expression of torpedo and rat acetylcholinesterase forms in  
transfected cells

Hermona Soreq (Hebrew University, Jerusalem, Israel)  
Human cholinesterase genes: molecular dissection and  
biomedical implications

**Discussant:** Victor Whittaker (Johannes Gutenberg University, Mainz, Germany)

---

**Session 5:           SYNTHESIS AND STORAGE OF ACH**

**Chair:**           **R. Jane Rylett** (Univ. Western Ontario, London, Canada)

**Speakers:**       **Paul Salvaterra** (City of Hope Res. Inst., Duarte, California, USA)  
Molecular genetic specification of cholinergic neurons

**Stanley Parsons** (University of Calif. Santa Barbara, California, USA)  
Acetylcholine transporter-vesamicol receptor pharmacology and structure

**Discussant:**     **Brian Collier** (McGill University, Montreal, Canada)

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**Session 6:           ACH TURNOVER AND RELEASE**

**Chair:**           **Oscar Scremin** (Wadsworth VA Medical Center, Los Angeles, USA)

**Speakers:**       **Konrad Löffelholz** (University of Mainz, Mainz, Germany)  
Choline, a precursor of acetylcholine and phospholipids in the brain

**Silvana Consolo** (Research Institute "Mario Negri", Milan, Italy)  
D1 and D2 dopamine receptors and the regulation of striatal  
IN VIVO acetylcholine release

**Discussant:**     **Stanislav Tuček** (Czech. Academy of Science, Prague, Czechoslovakia)

---

**Session 7:           MOLECULAR ASPECTS OF ACH RELEASE**

**Chair:**           **Georgina Rodriguez Lorez de Arnaiz** (University of Buenos Aires, Argentina)

**Speakers:**       **Maurice Israël** (CNRS, Gif-Sur-Yvette, France)  
Mediatophore performs the translocation step of the  
release mechanism

**Thomas Südhof** (Howard Hughes Med. Inst., Dallas, Texas, USA)  
 $\alpha$  - Latrotoxin receptor function in neurotransmitter release

**Discussant:**     **Yves Dunant** (University of Geneva, Switzerland)

---

**Session 8: TROPHIC INTERACTIONS**

**Chair:** Fred Gage (Univ. Calif. San Diego, La Jolla, California, USA)

**Speakers:** Lawrence Williams (Upjohn Company, Kalamazoo, USA)  
NGF affects the cholinergic neurochemistry and behaviour of aged rats

Franz Hefti (USC, Los Angeles, California, USA)  
Neurotrophin control of cholinergic neuron function  
and survival

**Discussant:** A. Claudio Cuello (McGill University, Montreal, Canada)

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**Session 9: ELECTROPHYSIOLOGICAL ASPECTS OF CHOLINERGIC MECHANISMS**

**Chair:** Alexander Karczmar (Loyola University, Illinois, USA)

**Speakers:** Kresimir Krnjević (McGill University, Montreal, Canada)  
Central cholinergic mechanisms and function

Malcolm Caulfield (Univ. Coll. London, London, England)  
Postsynaptic action of acetylcholine: the coupling of receptors and  
receptor subtypes to ion channels

**Discussant:** David McCormick (Yale Univ. Sch. of Med., Connecticut, USA)

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**Session 10: SECOND MESSENGERS**

**Chair:** Lowell Hokin (University of Wisconsin, Madison, USA)

**Speakers:** Richard Jope (University of Alabama, Birmingham, USA)  
Lithium selectively potentiates cholinergic activity  
in rat brain

Yoshihisa Kudo (Mitsubishi Kasei Inst. of Life Science, Tokyo, Japan)  
Characteristics of the changes in the cytosolic  $Ca^{2+}$  concentration during  
the stimulation of muscarinic receptor in hippocampal neurons

**Discussant:** Michael McKinney (Mayo Clinic, Jacksonville, Florida, USA)

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**Session 11: MODULATION OF INFORMATION**

**Chair:** Mircea Steriade (Laval Univ. Sch. of Med., Quebec City, Canada)

**Speakers:** Douglas Rasmusson (Dalhousie University, Halifax, Canada)  
Cholinergic modulation of sensory information

Charles Woody (UCLA, Los Angeles, USA)  
Cholinergic and glutamatergic effects on neocortical  
neurons may support rate as well as development of  
conditioning

**Discussant:** Adam Sillito (Institute of Ophthalmology, London, England)

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**Session 12: BEHAVIORAL ASPECTS OF CHOLINERGIC TRANSMISSION**  
**Forum for discussion:**

**Chair:** Toshitaka Nabeshima (Nagoya Uni. School Medicine, Nagoya, Japan)

**Discussants:** Stephen Dunnett (Univ. of Cambridge, Cambridge, England)  
Basal forebrain ACh and memory: discrepancies and a  
possible resolution

H. Christian Fibiger (Univ. of Brit. Columbia, Vancouver, Canada)  
Cholinergic mechanisms in learning and memory: implications for  
Alzheimer's disease

---

**Session 13: CHOLINERGIC INVOLVEMENT IN SLEEP AND AROUSAL**

**Chair:** J. Christian Gillin (UCSD, San Diego, USA)

**Speakers:** J. Allan Hobson (Harvard Med. Sch., Boston, USA)  
REM sleep induction by cholinergic microstimulation:  
locus and characteristics of short and long term  
enhancement sites in the pontine tegmentum of the cat

Mircea Steriade (Laval Univ. Sch. of Med., Quebec City, Montreal)  
Cholinergic blockage of intrinsically- and network-  
generated slow oscillations promotes waking and  
REM-sleep patterns in thalamic and cortical systems

**Discussant:** Barbara Jones (McGill University, Montreal, Canada)

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**Session 14: CLINICAL ASPECTS OF CHOLINERGIC PHARMACOLOGY**

**Chair:** Israel Hanin (Loyola Univ., Illinois, USA)

**Speakers:** Leslie Iversen (Merck Sharp & Dohme, Essex, England)  
Approaches to cholinergic therapy in Alzheimer's disease

Serge Gauthier (McGill Ctr. for Aging, Montreal, Canada)  
What have we learned from the THA trials to facilitate testing of new AChE inhibitors?

Albert Enz (Sandoz Pharma Ltd, Basle, Switzerland)  
Brain selective inhibition of cholinesterase: a new approach to the therapy for Alzheimer's disease

Robert Davis (Parke-Davis, Ann Arbor, U.S.A.)  
Subtype selective muscarinic agonists: potential therapeutic agents in Alzheimer's disease

**Discussant:** Ezio Giacobini (South Illinois Univ. Sch. of Med., Illinois, USA)

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**CLOSING LECTURE**

**Speaker:** Giancarlo Pepeu (Univ. of Florence, Florence, Italy)  
Overview and future directions on CNS cholinergic mechanisms



## ***INSTRUCTIONS FOR PSYMPIOSIUM SPEAKERS***

Speakers are requested to submit their slides to the projectionist in the conference room (Seigneurie 1) 30 minutes prior to the start of their session. Slide trays will be available in the conference room, where slides may be pre-screened if desired.

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## ***INSTRUCTIONS FOR POSTER PRESENTERS***

The poster session will be held in Seigneurie 2. Poster boards are numbered 1 - 96. The posters can be mounted, after 2 p.m., on Sunday July 26 on the poster board whose number corresponds to that of your poster abstract. Please refer to the first author index at the end of the Abstract Book for the appropriate poster abstract number. The posters will be on display for the duration of the meeting. Author attendance is requested during the Posters/Coffee breaks, immediately after or prior to the associated symposium session.

The posters should be removed by 9.00 p.m. Wednesday, July 29.

***SYMPOSIUM AND POSTER***

***ABSTRACTS***

## SESSION 1:

## ORGANIZATION OF CENTRAL CHOLINERGIC SYSTEMS

SYMPOSIUM ABSTRACTS

S1

**THE HISTOCHEMICAL REVELATION OF CENTRAL CHOLINERGIC SYSTEMS**

Barbara E. Jones, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada.

Since the presence of acetylcholine (ACh) and its synthetic enzyme was first demonstrated in the brain by biochemical assay some 50 years ago, the revelation of central cholinergic neurons has proceeded in a punctuated manner by technical advancements in histochemistry. Based upon the early developments in the 40's and 50's of the technique for staining the catabolic enzyme of ACh, acetylcholinesterase (AChE), Shute and Lewis described in the early '60s (1963) the distribution of putative cholinergic non-motor neurons and their major pathways within the brain. Visualizing AChE-stained fibers ascending from the brainstem into the forebrain and from the forebrain to the cerebral cortex, they hypothesized that the cholinergic neurons represented an ascending cholinergic reticular system, which corresponded to the ascending reticular activating system described in the early neurophysiological studies by Magoun and his colleagues (Starzl et al., 1951). By pharmacohistochemical modification and application of the AChE technique, multiple features of AChE stained neurons and their projections were elaborated through the '70's. With the development of immunohistochemical techniques and viable antibodies directed against the synthetic enzyme for ACh, choline acetyl transferase (ChAT), and their application in combination with modern neuroanatomical techniques, the full and correct details concerning the distribution of ACh-synthesizing neurons and their projections were finally uncovered in the '80's. Through this period, great impetus was lent to research into the organization of central cholinergic systems by the discovery of the relatively selective vulnerability of cholinergic neurons in Alzheimer's disease and the association of their degeneration with the major symptoms of this disease, which are related to a loss of vigilance, as would expectedly occur with the loss of a verified major component of the central reticular activating system.

S2

**CHOLINERGIC NEURONS IN THE RAT CENTRAL NERVOUS SYSTEM DEMONSTRATED BY *IN SITU* HYBRIDIZATION OF CHOLINE ACETYLTRANSFERASE mRNA**

Larry L. Butcher, Justin D. Oh, and Nancy J. Woolf University of California, Los Angeles, CA, 90024-1563, U.S.A.

Digoxigenin-labeled RNA probes and *in situ* hybridization histochemistry were used to examine choline acetyltransferase gene expression in the rat central nervous system. Hybridization signal was present only in brain sections processed with the antisense riboprobe; the sense probe did not yield labeling. Telencephalic neurons demonstrating the mRNA for the cholinergic synthetic enzyme were found in the caudate-putamen nucleus, nucleus accumbens, olfactory tubercle, Islands of Calleja complex, medial septal nucleus, vertical and horizontal limbs of the diagonal band, substantia innominata, nucleus basalis, and nucleus of the ansa lenticularis. Some somata evincing hybridization signal were observed the anterior amygdalar area, and an occasional such cell was seen in the basolateral and central amygdalar nuclei. Neurons in the cerebral cortex, hippocampus, and primary olfactory structures did not demonstrate hybridocytochemically detectable amounts of choline acetyltransferase mRNA. Thalamic cells were devoid of reactivity, with the exception of neurons located primarily in the ventral two-thirds of the medial habenula. A few somata labeled with riboprobe were found in the lateral hypothalamus, caudal extension of the internal capsule, and zona incerta. Neurons in the pedunculopontine and laterodorsal tegmental nuclei were moderately reactive, whereas cells of the parabigeminal nucleus exhibited very weak hybridization signal. No somata in the brainstem raphe nuclei, including raphe obscurus and raphe magnus, were labeled with riboprobe. In contrast, motor neurons of the cranial nerve nuclei demonstrated relatively large amounts of choline acetyltransferase mRNA. Putative cholinergic somata in the ventral horns and intermediolateral cell columns of the spinal cord also were labeled with riboprobe, as were a few cells around the central canal. We conclude that hybridocytochemistry with digoxigenin-labeled riboprobes confirms the existence of cholinergic neurons (i.e., those that synthesize and use acetylcholine as a neurotransmitter) in most of the neural regions deduced to contain them on the basis of previous histochemical and immunocytochemical data. Notable exceptions are the cerebral cortex and hippocampus, which do not possess neurons expressing detectable levels of choline acetyltransferase mRNA.

## SESSION 1: ORGANIZATION OF CENTRAL CHOLINERGIC SYSTEMS

### SYMPOSIUM ABSTRACTS

#### S3 CHOLINERGIC SYSTEMS: ABNORMALITIES IN NEUROLOGICAL DISEASE AND PROSPECTS FOR THERAPY

D.L. Price<sup>1,2</sup>, R.E. Clatterbuck<sup>2</sup>, D. Olton<sup>3</sup>, AND V.E. Koliatsos<sup>1,2</sup>;  
<sup>1</sup>Departments of Pathology and Neurology, <sup>2</sup>Neuropathology Laboratory, The Johns Hopkins University School of Medicine, <sup>3</sup>Department of Psychology, The Johns Hopkins University, Balto., MD, USA.

Populations of cholinergic neurons show specific vulnerabilities in several human neurological disorders. Lower motor neurons degenerate in amyotrophic lateral sclerosis; neurons of Onuf's nucleus, which influence potency, are destroyed in the Shy-Drager syndrome; and basal forebrain cholinergic neurons degenerate in Alzheimer's disease. This concept is illustrated by investigations showing that nerve growth factor (NGF) acts on basal forebrain cholinergic neurons, which synthesize p75 NGF receptor and p140<sup>trkA</sup>, which, together, comprise the high-affinity NGF receptor. When axons of these neurons are transected by lesions of the fimbria-fornix in rats and monkeys, neurons of the medial septum undergo retrograde degeneration; the administration of NGF prevents retrograde degeneration of these neurons (at least for short intervals). Moreover, several studies, including those from our laboratories, indicate that cholinergic-dependent memory deficits in subsets of aged rats respond to NGF. The identification of trophic influences on subsets of nerve cells vulnerable in specific diseases may allow the design of biological therapies to treat these human neurological disorders.

#### S4 ANATOMICAL ORGANIZATION OF CHOLINERGIC SYSTEMS

Bruce H. Wainer,<sup>1</sup> Teresa L. Steininger,<sup>1</sup> Jeffrey H. Kordower,<sup>2</sup> and Melanie Burke-Watson<sup>2</sup> <sup>1</sup>The University of Chicago and <sup>2</sup>Rush University, Chicago, IL

A considerable body of evidence has demonstrated an important role for non-motor central cholinergic transmission in cognition and behavioral state control. The application of immunohistochemistry and *in situ* hybridization for the visualization of cholinergic neurons, and the refinement of axonal tracing techniques, has allowed the identification of cholinergic systems that mediate the behavioral effects of acetylcholine. These systems include the magnocellular basal forebrain and mesopontine tegmental cholinergic groups. Cholinergic neurons in the basal forebrain are involved in memory and selective attention and are distributed across several classically defined nuclei including the medial septum, the nucleus of the diagonal band of Broca, and the nucleus basalis of Meynert. These neurons provide cholinergic innervation of the neocortex and hippocampus and are responsive to neurotrophins, including nerve growth factor (NGF), which are synthesized in target areas. These cell groups are vulnerable in Alzheimer's disease, and the potential of trophic factor therapy has generated considerable interest in the mechanisms of neurotrophin action. The cholinergic neurons of the mesopontine tegmentum are contained within the pedunculopontine tegmental (PPT) and laterodorsal tegmental (LDT) nuclei. Ascending projections of the PPT and LDT to the basal forebrain, hypothalamus, and thalamus, as well as descending projections to the pontine and medullary reticular formation may be involved in behavioral arousal and the modulation of sleep states. Recent data suggests that afferents to these cell groups originate in heterogeneous brain regions that have been implicated as modulators of behavioral state including the dorsal raphe nucleus, lateral hypothalamus, and periaqueductal gray. While putative trophic factors for motor (ciliary neurotrophic factor) and basal forebrain (neurotrophin family) cholinergic cell groups have been identified, target-derived trophic support for the brainstem cell groups has yet to be elucidated.

## SESSION 1: ORGANIZATION OF CENTRAL CHOLINERGIC SYSTEMS

### POSTER ABSTRACTS

- P1 **ULTRASTRUCTURAL ASPECTS OF THE ACETYLCHOLINE (ACh) INNERVATION IN ADULT RAT PARIETAL CORTEX.** D. Umbriaco, K.C. Watkins, L. Descarries, C. Cozzari and B.K. Hartman. CRSN (Départements de pathologie et de physiologie), Université de Montréal, Montréal (Canada); Istituto Biologia Cellulare CNR, Roma (Italy), and Department of Psychiatry, University of Minnesota, Minneapolis (USA).

More than 800 ACh axon terminals (varicosities) from the different layers of adult rat parietal cortex (Par1) were examined in serial sections for electron microscopy, after immunostaining with monoclonal antibodies against purified rat brain choline acetyltransferase. These varicosities were all photographed across their entire volume, at a final magnification of 36 000 X (average of 11 pictures per varicosity). Several were reconstructed in three dimensions, using the ICAR 80.8 (ISG Technologies) volume investigation station from Nissei Sangyo Canada. The mean maximal transverse diameter of these varicosities was similar in every layer ( $0.5 \pm 0.15 \mu\text{m}$  s.d.). Both large and small varicosities could be observed on the same axons. In each layer, a relatively low proportion exhibited a synaptic membrane differentiation (9% in layer I; 14% in II-III; 12% in IV; 22% in V; 15% in VI), for a I-VI average of 14%. These 118 synaptic junctions were almost invariably symmetrical (98%). A majority were found on dendritic branches (81%), some on spines (23%) and none on cell bodies. There were only 4 varicosities with dual junctions, and the 2 asymmetrical junctions were on spines in layers I and II-III. On the whole, junctional varicosities were slightly larger than the non junctional, even though both types were present on the same fibers. This study demonstrates that the ACh innervation is mostly non junctional in every layer of adult rat parietal cortex. ACh receptive elements will need to be visualized in order to identify all functional targets of such an innervation. [Supported by the FCAR and grants MT-3544 (MRC) and NS 12311].

- P2 **CATECHOLAMINERGIC/CHOLINERGIC INTERACTION IN THE BASAL FOREBRAIN**  
Laszlo Zaborszky, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

Immunocytochemical double-labeling techniques were used at the light and electron microscopic levels to investigate whether dopamine- $\beta$ -hydroxylase (DBH) and tyrosine hydroxylase (TH)-containing axons contact basal forebrain cholinergic projection neurons (BFC). DBH- and TH-positive fibers and terminals were found in close proximity to cholinergic neurons (as revealed using an antibody against choline acetyltransferase) throughout extensive basal forebrain areas, including the vertical and horizontal limb of the diagonal band nuclei, the sublenticular substantia innominata (SI), ventral pallidum and globus pallidus (GP). Parallel experiments at the electron microscopic level confirmed synaptic contacts between DBH-positive terminals and distal cholinergic dendrites in the SI. DBH-positive boutons are usually large, containing clear and dense core vesicles and the synapses were always of the asymmetric type with prominent postsynaptic subjunctional bodies. Preliminary experiments following PHA-L injection in the locus coeruleus suggest that at least part of the noradrenaline innervation of BFC neurons originates in the locus coeruleus. Cholinergic cells in the ventromedial GP and internal capsule appeared to be contacted by tyrosine hydroxylase-positive but not DBH-positive fibers, suggesting dopaminergic input to cholinergic neurons in these regions. TH axons in these two regions establish symmetric synapses with BFC cells. While a few TH-positive boutons contacted the cell body, the majority of such synapses were found on dendrites. Supported by USPHS grants No. 23945 and 300024.

## SESSION 1:

## ORGANIZATION OF CENTRAL CHOLINERGIC SYSTEMS

### POSTER ABSTRACTS

- P3 COLOCALIZATION OF CHOLINE ACETYLTRANSFERASE (ChAT) AND VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) IN RAT INTRACORTICAL NEURONS: A DOUBLE IMMUNOFLOUORESCENCE STUDY. A. Chédotal,<sup>1</sup> L. Descarries,<sup>2</sup> C. Cozzari<sup>3</sup> and E. Hamel<sup>1</sup>. <sup>1</sup>Montreal Neurological Institute, McGill University, <sup>2</sup>Université de Montréal, Montréal, Québec, Canada and <sup>3</sup>Istituto Biologia Cellulare CNR, Roma, Italy.

Regulation of higher integrative functions and of intracortical cerebral blood flow has been associated with the cholinergic innervation of the cerebral cortex. Such innervation, however, has a dual origin: the basal forebrain (BF) primarily and, to a smaller extent, the intrinsic cortical bipolar neurons. The latter have been claimed to be the sole source of cortical VIP (Eckenstein FP et al., 1988, *Neuroscience* 25:457-474). In order to evaluate if intrinsic cholinergic terminals could be distinguished from those of the BF by their colocalization with VIP, a double immunofluorescent labeling of ChAT and VIP cortical neurons was performed. Coronal cortical brain sections were incubated overnight with a mixture of ChAT (MCAT-1C, Cozzari et al., Soc. Neurosci. Abstr. 16: 200, 1990) and VIP (Peninsula) antibodies. ChAT and VIP were simultaneously visualized with fluorescein (FITC) and streptavidin Texas Red, respectively, in order to determine the proportions of ChAT, VIP and ChAT/VIP neurons. To ascertain the sensitivity of the fluorescence technique, sections were sequentially processed for ChAT with the avidin-biotin method and DAB-nickel as a chromogen, and then for VIP with FITC. Irrespective of the cortical area examined (n = 1686 neurons), VIP immunofluorescent cells accounted for the largest proportion of cortical immunostained neurons ( $58.8 \pm 1.7\%$ ), followed by ChAT-positive cells ( $27.8 \pm 1.7\%$ ) with only  $13.2 \pm 1.3\%$  of perikarya being double-labeled for VIP and ChAT. Similar proportions were obtained for VIP (62%) and ChAT (38%) when neurons were labeled sequentially (n = 1504). Of the cortical ChAT-immunostained cells (n = 693), 32% colocalized VIP whereas only 18% of VIP neurons (n = 1216) also contained ChAT. These results clearly show that a majority of VIP neurons do not colocalize ChAT. Therefore, it appears that VIP alone cannot be used as a marker of intracortical cholinergic neurons and terminals. Such results emphasize the need for separate assessments of VIPergic and cholinergic structure and function within the cerebral cortex. Supported by the MRC of Canada.

- P4 POSITRON EMISSION TOMOGRAPHIC STUDIES OF CENTRAL CHOLINERGIC NERVE TERMINALS. L. Widén, L. Eriksson, M. Ingvar, S.M. Parsons, G.A. Rogers and S. Stone-Elander. Dept. of Clinical Neurophysiology, Karolinska Hospital, and Karolinska Pharmacy, Stockholm, Sweden, and Dept. of Chemistry and Neuroscience Research Institute, University of California, Santa Barbara, USA.

The aim of this study was to develop a quantitative method for positron emission tomographic (PET) studies of central cholinergic nerve terminals. We first synthesized an F-18-labeled analogue of vesamicol ([F-18]FMV) that binds with high affinity to synaptic vesicles from Torpedo electric organ. It was evaluated in vivo in rats and monkeys by PET. In rats, the tracer was rapidly cleared from the blood and highly extracted into the brain, where it was specifically and essentially irreversibly bound. In monkeys, a specific binding of the tracer was observed in brain regions known to contain cholinergic nerve terminals. Protection of the binding sites by preinjection of non-labeled (-)vesamicol prevented the cerebral binding of [F-18]FMV to a high affinity site in both species.

Although the binding characteristics of [F-18]FMV were promising, its high lipophilicity made separation of the receptor characteristics from blood flow information difficult. Therefore, an analogue of the aminobenzovesamicol family, N-ethyl [F-18]fluoroacetylaminobenzovesamicol, NEFA, was synthesized and evaluated in rats and monkeys as above. It proved to be less lipophilic than [F-18]FMV. Its affinity for the vesamicol receptor was higher, the binding was protectable and the unspecific binding lower. [F-18]NEFA will be tested in healthy human subjects and in patients suffering from Alzheimer's disease.

## SESSION 2: CNS DISTRIBUTION OF RECEPTORS

### SYMPOSIUM ABSTRACTS

#### S5 CNS DISTRIBUTION OF CHOLINERGIC RECEPTORS - SOME QUESTIONS FROM A CLINICAL NEUROSCIENTIST

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During the last decade a complex heterogeneity of muscarinic and nicotinic receptors has emerged based upon ligand-binding characterization and gene cloning in molecular biology. The functional role of these subtypes in the CNS as well as their cellular localisation is however mainly unknown and comparative interpretations from lesion experiments in animals to postmortem studies in neurodegenerative disorders are not easily made. For instance in the rat, a thalamic lesion is followed by a marked decrease in cortical nicotinic sites, indicating their localisation on thalamo-cortical projections, while in Alzheimers disease similar reductions are commonly considered due to degeneration of cholinergic projections from the basal forebrain. How to explain the conflicting reports on unchanged, decreased or increased number of muscarinic receptors in certain brain regions with age or in states of neurodegeneration? Different neurotransmitter binding sites are expressed on glial cells in culture. To what extent do changes in receptor density as demonstrated in homogenates or in autoradiographic sections reflect glial mechanisms?  $^{11}\text{C}$ - or  $^{18}\text{F}$ -labelled muscarinic antagonists, nicotine as well as ligands for the vesicular transporter are presently used for positron emission tomography in man and non-human primates. How do the results agree with postmortem studies and will the turnover rates of different cholinergic receptors in the CNS be estimated?

#### S6 CNS NICOTINIC RECEPTORS: LOCALIZATION AND RELATION TO CHOLINERGIC TRANSMISSION

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An unknown number of subtypes of nicotinic ACh receptor (nAChR) are expressed in the CNS. One prominent population is labelled with high affinity by  $^3\text{H}$ -nicotine; another is labelled by  $^{125}\text{I}$ - $\alpha$ -bungarotoxin. There is abundant evidence for brain nAChRs that gate inward cation currents, promoting increased neuronal firing and transmitter release. However, there is also a growing realization that certain nAChRs, particular those that bind  $\alpha$ -bungarotoxin, may trigger other cellular responses. Several major neuronal systems appear to express nAChRs at the level of cell bodies and/or dendrites and also at the level of terminals. Of particular interest, the mesolimbic dopamine system can be directly activated by nicotine and this action appears to contribute to the reinforcing effects of the drug. It will be important to determine which nAChR subtype(s) are involved, in order to direct the development of pharmacotherapies for nicotine dependence. The limited evidence available indicates a synaptic location for nAChRs that bind  $^{125}\text{I}$ - $\alpha$ -bungarotoxin; no comparable data exist for those labelled with  $^3\text{H}$ -nicotine. Moreover, although CNS nAChRs are clearly cholinergic, it is unclear how many are targets for endogenous ACh. Several candidate neuronal pathways will be illustrated that possibly employ nicotinic cholinergic transmission.

## SESSION 2: CNS DISTRIBUTION OF RECEPTORS

### SYMPOSIUM ABSTRACTS

- S7 **COMPARATIVE DISTRIBUTION OF MULTIPLE MUSCARINIC RECEPTOR SUB-TYPES IN THE MAMMALIAN BRAIN:** Quirion R., Araujo D. & Aubert I. Douglas Hospital Research Centre, and Department of Psychiatry, and Pharmacology and Therapeutics, Faculty of Medicine, McGill University, Montreal, Quebec, Canada H4H 1R3.

Molecular biology approaches have clearly demonstrated the existence and expression (mRNAs) of five ( $m_1$ - $m_5$ ) sub-types of muscarinic receptors in the CNS. It is still difficult to precisely assess the comparative distribution of the respective receptor protein due to the lack of highly sub-type selective radioligands and/or fully characterized receptor antibodies. However, recent progress has been made in these directions and will be reviewed here. A comparative investigation of the autoradiographic localization of specific receptor binding sites for the universal ligand [ $^3H$ ]QNB, and that of putative  $M_1$  ([ $^3H$ ]pirenzepine),  $M_2$  ([ $^3H$ ]AF-DX116, [ $^3H$ ]AF-DX384) and  $M_3$  ([ $^3H$ ]4-DAMP) sites revealed the differential distribution of these three classes of muscarinic receptors in the neonatal, adult and aged mammalian brain. For example, while  $M_1$  sites are concentrated in the frontoparietal cortex and the hippocampal formation,  $M_2$  labelling is most prominent in various thalamic and brainstem nuclei.  $M_3$  sites are apparently most concentrated in the dentate gyrus and the CA<sub>1</sub> sub-field of the hippocampus. Interestingly, while putative  $M_2$  sites revealed using [ $^3H$ ]acetylcholine are mostly distributed as seen with the antagonists, their localization in the septo-hippocampal pathway is clearly different suggesting the recognition by agonists and antagonists of different receptor sub-types in this projection. A comparison with the discrete localization of choline acetyltransferase (ChAT) and [ $^3H$ ]hemicholinium-3, a marker of the high affinity choline uptake, suggests the preferential association of  $M_2$  (but not  $M_1$ ) sites with cholinergic perikarya and nerve terminals. This concept is supported further by functional release and behavioral data, although the putative role, in that regard, of the  $m_4$  and/or  $m_5$  sub-types will have to await the development of related selective probes. (Supported by the MRCC and the Alzheimer Society of Canada).

- S8 **CNS DISTRIBUTION OF RECEPTORS : RECENT PROGRESS IN RECEPTOR VISUALIZATION.** J.M. Palacios, G. Mengod, T. Vilaró, Research Institute, Laboratorios Almirall, Cardener, 68-74, 08024 Barcelona (Spain) and C.I.D. Consejo Superior Investigaciones Científicas, Jordi Girona, 18-26, 08034 Barcelona (Spain).

Our understanding of the regional and cellular distribution of cholinergic receptors in the brain has been advanced considerably by the isolation of the gene coding for the 5 different muscarinic cholinergic receptors, the characterization of more subunits of the nicotinic receptors, the development of several new ligands with different selectivities for these receptors and by the production of antibodies against the different receptor proteins involved. The speakers in this session will review these advances.

Our own work has been directed towards the detailed delineation of the cellular and regional distribution of the 5 different muscarinic receptors, in the rat brain initially, using *in situ* hybridization combined with classical receptor autoradiography. We have identified all 5 receptor subtypes in the rat brain. Some brain structures express most or all of the subtypes, in a second group of structures a single subtype predominates although others are present and finally a further group seems to express a single subtype only.

We have also compared the pharmacological characteristics of the binding of several radiolabelled ligands in these different areas and delineated conditions for selective labelling of subpopulations of receptors. Furthermore, cellular localization studies have indicated their pre- or post-synaptic localizations with respect to cholinergic neurons and other chemically identified neuronal populations.



## SESSION 2: CNS DISTRIBUTION OF RECEPTORS

### POSTER ABSTRACTS

#### P5 REGIONAL DISTRIBUTION OF MUSCARINIC RECEPTOR SUBTYPES IN MACAQUE HIPPOCAMPAL FORMATION BY IN VITRO RECEPTOR AUTORADIOGRAPHY

Maria Giuliana Vannucchi+\*, Giancarlo Pepeu+ , Patricia Goldman-Rakic\*

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The distribution of different muscarinic receptor subtypes was investigated in the hippocampus of rhesus monkey (*Macaca mulatta*) by using techniques of in vitro receptor autoradiography. Binding sites were labeled with (3H)-Pirenzepine (PZ) and (3H)-AFDX384 and nonspecific binding was determined in the presence of 10  $\mu$ M atropine. (3H)-PZ bound with high affinity to one class of receptors that were highly concentrated in the dentate gyrus, CA4 and CA3. The labeling was faint in CA2 and increased in CA1. Moderate binding was observed in the pre-subiculum but it was almost undetectable in the subiculum. (3H) AFDX384 binding sites in the hippocampus proper by contrast were concentrated in CA4, CA3 and also CA2 while they were hardly seen in the dentate gyrus and CA1. Unlike (3H)-PZ, AFDX384 binding was intense in the subiculum; the border between the hippocampus proper and subiculum was accordingly sharp, while further labeling in the presubiculum was faint. These results suggest that the two labeled compounds bind to two different classes of muscarinic receptors that partially overlap in the hippocampus proper but show a striking complementarity in the subicular cortices.

#### P6 HETEROGENEOUS MUSCARINIC RECEPTOR SUBTYPES IN BOVINE CEREBRAL CORTICAL MICROVESSELS. D.G. Linville and E. Hamel, Laboratory of Cerebrovascular Research, Montreal Neurological Institute, McGill University, Montréal, Québec, Canada H2A 2B4

Acetylcholine is involved in the regulation of cortical cerebral blood flow through muscarinic receptors possibly located in part on intraparenchymal blood vessels. However, muscarinic receptors are highly heterogeneous and five genes encoding different receptors have recently been identified and may be present in cerebral blood vessels. In the present study, the subtype(s) of muscarinic receptors located on microvessels isolated from bovine cerebral cortex were pharmacologically characterized by competition studies against the binding of [<sup>3</sup>H]-N-methylscopolamine ([<sup>3</sup>H]-NMS). Microvessels corresponded primarily to large vessels containing smooth muscle and were highly enriched in marker enzymes alkaline phosphatase and  $\gamma$ -glutamyl transpeptidase (18 and 22-fold over cortical values, respectively). Specific binding of [<sup>3</sup>H]-NMS was saturable and of high affinity with a binding capacity ( $B_{max}$ ) of  $29.8 \pm 3.9$  fmol/mg protein and  $K_D$  of  $183 \pm 57$  pM. Antagonists with established affinity at the various muscarinic receptors inhibited the binding of [<sup>3</sup>H]-NMS to microvascular membranes with different potencies. Pirenzepine delineated two populations of sites, one with high affinity ( $pK_{DH} = 8.25 \pm 0.17$ ) which corresponded to approximately 37% of the sites, and a second site with low affinity ( $pK_{DL} = 6.88 \pm 0.27$ ). All other antagonists were best fitted (LIGAND) with binding to a single site with the following potencies ( $pK_D$ ): HHSiD ( $7.15 \pm 0.27$ ); AF-DX 384 ( $7.33 \pm 0.09$ ); AQ-RA 741 ( $7.41 \pm 0.21$ ); methoctramine ( $7.41 \pm 0.23$ ) and DAU 6202 ( $8.16 \pm 0.15$ ). Correlation analysis performed between the antagonists vascular potency and their published affinity ( $pK_i$ ) at either pharmacological (Ladinski and Schiavi, Soc. Neurosci. Abst. 16:1061, 1990) or cloned (human or rat) muscarinic receptors ( $m_1$ - $m_5$ ) expressed into CHO-cells (Buckley et al., Mol. Pharm. 35:469, 1989; Dörje et al., JPET 256:727, 1991 and H.N. Doods, personal communication) identified a population of  $m_1$  receptors and excluded the presence of  $m_2$  and  $m_3$  subtypes. Both the  $m_1$  and  $m_4$  subtypes remain as possible candidates for the low affinity site identified with pirenzepine. These results support the multiplicity of muscarinic receptors in bovine intracortical microvessels and suggest an important role of the  $m_1$  (and possibly  $m_2$  and/or  $m_4$ ) subtype in microvascular vasomotor and/or nonvasomotor functions. Supported by the MRC of Canada, the FRSQ and Dr. Karl Thomae GmbH.

## SESSION 2: CNS DISTRIBUTION OF RECEPTORS

### POSTER ABSTRACTS

- P7 EVIDENCE FOR THE PRESENCE OF A PHARMACOLOGICAL M4 MUSCARINIC RECEPTOR SUBTYPE IN RAT BRAIN COUPLED NEGATIVELY TO ADENYLATE CYCLASE AS DETECTED BY A NEW ANTAGONIST DAU 6202. H. Ladinsky<sup>1</sup>, M. Zambelli<sup>2</sup>, C. La Porta<sup>2</sup>, M. Parenti<sup>3</sup>, A. Zocchetti<sup>3</sup>, M. Turconi<sup>1</sup>, S. Consolo<sup>2</sup> and G.B. Schiavi<sup>1</sup>. <sup>1</sup>Department of Biochemistry and Molecular Pharmacology, Boehringer Ingelheim Italia, Milan 20139 Italy, <sup>2</sup>Mario Negri Institute, Milan 20157 Italy and <sup>3</sup>Department of Pharmacology, University of Milan, Milan, Italy.

Inhibition of (<sup>3</sup>H)PZ binding to cortical M1 muscarinic receptors (nAChRs) and (<sup>3</sup>H)NMS binding to cardiac M2, glandular M3 and brain regional mAChRs was studied with a new antimuscarinic agent, DAU 6202 (4-hydroxy-3-(tropyl)oxycarbonyl-3,4-dihydro-1 H-quinazoline-2-one). DAU 6202 bound to the mAChRs with K<sub>D</sub>s of (nM): 1.8, M1; 4.3, M3; 204, M2. In ventral hippocampus, a shallow inhibition curve was seen suggesting that DAU 6202 bound to a heterogeneous population of sites. Computer analysis showed that DAU 6202 bound 67% of total sites with a K<sub>D</sub> of 3 nM and 33% with a K<sub>D</sub> of 42 nM. The 3 nM site likely represents a mixture of M1 and M3 mAChRs; the 42 nM site, non M1, non M2, and non M3 appears to represent an M4 site. M2 mAChRs were not detected by this compound. From the affinity of DAU 6202 (K<sub>b</sub>, 2.5 ± 1.0 nM) to antagonize 1 mM carbachol stimulated [<sup>3</sup>H]inositol phosphates accumulation in ventral hippocampus, it was suggested that this response was activated by the M1 and M3 receptors. The inhibition of forskolin stimulated cAMP accumulation by 1 mM carbachol in the area was antagonized by DAU 6202 (K<sub>b</sub>, 37 ± 0.7 nM) suggesting involvement of the M4 receptors. In other brain regions, too, DAU 6202 gave shallow inhibition curves revealing M4 sites of major proportions, i.e. cortex, 38%, striatum, 69%; olfactory bulb, 46%; hypothalamus, 56% of total sites.

### SESSION 3: MOLECULAR ASPECTS OF RECEPTORS

#### SYMPOSIUM ABSTRACTS

- S9 **MOLECULAR ASPECTS OF ACETYLCHOLINE RECEPTORS.** Herbert Ladinsky, Boehringer Ingelheim Italia, Milan, Italy.

Until recently, the study of receptors was almost exclusively undertaken using physiological and pharmacological methods. In the case of the acetylcholine receptor, two main classes could be defined pharmacologically, the muscarinic and nicotinic receptors. At the molecular level little was known of either their structure or of the mechanisms by which their activation resulted in cellular responses. The application of the radioligand binding technique has provided the necessary tool in receptor research for elucidating molecular properties of the receptors. For the nicotinic receptor, the discovery of powerful ligands for it in the form of certain snake venoms, or  $\alpha$ -toxins, provided the requisites that eventually led to the isolation, for the first time, of a transmitter receptor and then to the cloning of the receptor from the Torpedo electric organ. Analogously, the discovery of pirenzepine and the initial elucidation of the muscarinic receptors into M1 and M2 subtypes, provided the impetus to clone the first muscarinic receptor (m1) from pig cortex. We now know from work spanning the past five years that the muscarinic receptor gene family encodes five member proteins (m1-m5) while the neuronal and muscle nicotinic gene families consist of multiple subunits, currently ten in the former. One puzzle nowadays is, more so than as to how many receptors are there, is simply the question of why there are so many subtypes or subunits of the receptor. Possibly, the study of receptor function will demonstrate a functional diversity and suggest physiological explanations for the multiplicity. Exploitation of such knowledge must lead to the discovery of second generation selective drugs that could be much more effectively directed than at present.

#### FUNCTIONAL DIVERSITY OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS

- S10 Jim Patrick, Phillipe Seguela, Steve Vernino, Santosh Helekar, Mariano Amador, Jacques Wadiche, and John A. Dani

Division of Neuroscience and Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, Texas 77030

The neuronal nicotinic acetylcholine receptor gene family has ten members that form either homo-oligomeric or hetero-oligomeric ligand gated ion channels. Although the total number of combinations of possible receptors is large, only a few have been demonstrated in the oocyte expression system. However, those that have been expressed differ from each other in several important ways. They are activated and inhibited by different classes of ligands, they have different single channel properties, and they have different permeabilities. We have shown that one neuronal nicotinic receptor ( $\alpha 3/\beta 4$ ) is markedly more permeable to calcium ions than is the muscle type nicotinic receptor. Furthermore, this receptor is modulated by calcium acting on the external domain of the molecule; increasing calcium both increases whole cell current and decreases the single channel current. Thus, these receptors can respond to extracellular calcium and affect cytoplasmic processes by altering intracellular calcium. The homo-oligomeric receptor formed from the  $\alpha 7$  gene product has a greater permeability to calcium than do the other neuronal nicotinic acetylcholine receptors. This suggests that the  $\alpha 7$  receptor is similar to the NMDA receptor in its permeability to calcium and may play a similar role in regulating calcium dependent cytoplasmic mechanisms that are important in neuronal plasticity and neurotoxic cell death.

### SESSION 3: MOLECULAR ASPECTS OF RECEPTORS

#### POSTER ABSTRACTS

P8 **m1, m2 AND m4 MUSCARINIC RECEPTORS COUPLE TO INWARDLY RECTIFYING POTASSIUM CONDUCTANCES**

S.V. Penelope Jones

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Muscarinic receptors differentially regulate inwardly rectifying potassium currents. Muscarinic receptor subtypes m2 and m4 increase, while m1 reduces the inward potassium current. In ArT-20 cells, northern blot analysis revealed that they express the m4 muscarinic receptor subtype endogenously. These cells also express a Cs sensitive time-dependent inward potassium conductance. RBL-2H3 cells on the other hand express a time-independent, fast activating, Cs and Ba sensitive inward potassium conductance. RBL 2H3 cells do not express muscarinic receptors endogenously, but have been stably transfected with the m2 and m4 muscarinic receptors. In both ArT-20 and RBL 2H3 cells, m2 and m4 stimulate a fast activating, time-independent inwardly rectifying potassium conductance. This increase in potassium current can be inhibited by preincubation with 100ng/ml pertussis toxin overnight. Intracellular application of GTP $\gamma$ S initially enhanced and then inhibited muscarinic stimulation of the inward potassium currents. Thus, these responses appear to be mediated by a pertussis toxin sensitive G-protein. In m1-transformed RBL-2H3 cells, stimulation of the receptor with acetylcholine resulted in a reduction of the inward potassium current. This was accompanied by the activation of outward potassium current and exocytosis.

P9 **CHIMERIC M<sub>1</sub>/M<sub>2</sub> MUSCARINIC RECEPTORS: CORRELATION OF LIGAND SELECTIVITY AND FUNCTIONAL COUPLING WITH STRUCTURAL MODIFICATIONS.** J. Lai, L. Nunan, S.L. Waite, S.W. Ma, J.W. Bloom, W.R. Roeske and H.I. Yamamura. Depts. Pharmacology and Internal Medicine, University of Arizona HSC, Tucson, AZ 85724.

Five mammalian muscarinic acetylcholine receptor subtypes, m1-m5, are distinguished by their primary structure. The proposed secondary structure for these polypeptides consist of seven transmembrane, hydrophobic domains (I-VII), three extracellular domains (o) and N terminal, and three intracellular domains (i) and C terminal. *In vitro* expression of the m1 and m2 receptors demonstrated that the properties of these two subtypes correlate well with the pharmacologically defined M<sub>1</sub> and M<sub>2</sub> subtypes in mammalian tissues, respectively. We hypothesize that the selectivity of a number of antimuscarinics and the agonist carbachol for the receptor subtypes are due to the structural diversity among the ligand binding domains of the subtypes. A comparative analysis of structural diversity between the M<sub>1</sub> and M<sub>2</sub> receptors was achieved by chimeric recombination of the two receptors followed by functional expression *in vitro*. The binding affinities of a number of ligands for these chimeric receptors were compared with their affinities for the M<sub>1</sub> and M<sub>2</sub> receptors. The tricyclic compounds, namely pirenzepine (PZ), AF-DX 116 and himbacine, shared a binding site between domains VI and VII. However, the selective interaction of PZ with M<sub>1</sub>, and AF-DX 116 and himbacine with M<sub>2</sub> involved different structural regions. The high affinity binding for 4-DAMP and HHSiD was confined to within loop o2 and domains V and VI. These results support the hypothesis that the ligands' stereochemical features are critical in their optimal alignment, thus high affinity interaction within the ligand binding pocket. The cytoplasmic i3 loop modulated the binding of carbachol such that receptors which contained the i3 domain from the M<sub>2</sub> receptor exhibited a single high affinity state whereas those with the i3 domain from the M<sub>1</sub> receptor had an additional low affinity state for the agonist. The i3 regions were essential for the differential functional coupling of the M<sub>1</sub> and M<sub>2</sub> receptors to second messenger systems, however, additional upstream regions appeared to be essential for a potent and efficacious activation of phospholipase C by the M<sub>1</sub> receptor. Supported by AHA, USPHS and ADCRC.

### SESSION 3: MOLECULAR ASPECTS OF RECEPTORS

#### POSTER ABSTRACTS

- P10 THE USE OF MURINE FIBROBLAST CELL LINES TRANSFECTED WITH CLONED MUSCARINIC RECEPTORS THAT EXHIBIT STABLE EXPRESSION: MODELS FOR THE STUDY OF MUSCARINIC RECEPTOR MECHANISMS. William R. Roeske, Eva Varga, Hong Bing Wei, Ildiko Kovacs, Ichiro Sora, Sue Waite, Josephine Lai, and Henry I. Yamamura. Departments of Pharmacology and Internal Medicine, University of Arizona Health Science Center, Tucson, Arizona 85724.

Murine fibroblast cell lines that have been transfected with each of the five rat muscarinic receptor genes (m1-m5) were used in this study. In addition, selected human muscarinic genes, muscarinic chimeric receptors and other mutants have also been cloned and expressed in this cell line. Using [<sup>3</sup>H](-)MQNB to label receptors, we have studied drug selectivity and specificity. We have also studied coupling mechanism using these clones. We have studied homologous down-regulation of the M<sub>1</sub> and M<sub>2</sub> muscarinic receptors in transfected fibroblast B82 cells. The cells were pretreated with (+)-cis-methyldioxolane (CD), carbachol (CCh), (-)YM796 or atropine for up to 24 hours. The muscarinic full agonist CD resulted in the loss of the M<sub>2</sub> binding sites more rapidly than that of the M<sub>1</sub> binding sites measured by [<sup>3</sup>H](-)MQNB binding. After 24 hr exposure of the cells to CD, the densities of the M<sub>1</sub> and M<sub>2</sub> receptors decreased 76-78%. Both the M<sub>1</sub> receptor mediated phosphoinositide hydrolysis and the M<sub>2</sub> receptor mediated inhibition of forskolin-stimulated cAMP formation were attenuated. We have studied chimeric constructs for analysis of domains for drug selectivity, specificity and coupling mechanisms. We have compared the human M<sub>2</sub> receptors (cardiac subtype) with the rat M<sub>2</sub> receptors expressed in the same cell line in order to assess drug specificity, selectivity and coupling mechanisms. Both clones exhibited the same order of potency for the selective agonists and antagonists studied. Initial studies with carbachol reversal of forskolin-stimulated adenylyl cyclase showed no differences in coupling for these two receptors. Conclusions: 1) Murine fibroblast cell lines that are transfected with the muscarinic receptors and exhibit stable expression are useful for the determination of agonist and antagonist selectivity and specificity; 2) these models can be conveniently used for the study of homologous down-regulation; and 3) in contrast with the literature, there are no species differences in the transfected M<sub>2</sub> receptors. Supported by AHA and USPHS grants.

- P11 A COMPARISON OF  $\alpha 3/\beta 2$  AND  $\alpha 4/\beta 2$  NICOTINIC ACETYLCHOLINE RECEPTORS AT THE SINGLE CHANNEL LEVEL. Michel Paquet and Ellis Cooper. Dept. of Physiology, McGill University, Montréal, Québec. H3G 1Y6.

Neuronal nicotinic acetylcholine receptors (nAChRs) are made up of alpha ( $\alpha$ ) subunits that bind ligand and non-alpha ( $\alpha$ ), or beta ( $\beta$ ), subunits that do not bind ligand. To date, seven different  $\alpha$  subunits ( $\alpha 2$ - $\alpha 8$ ) and three different  $\beta$  ( $\beta 2$ - $\beta 4$ ) subunits have been identified. Oocyte expression studies have shown that co-expression of mRNA encoding an  $\alpha$  and a  $\beta$  subunit direct the assembly of functional nicotinic receptors in oocyte membranes, and combinations of different  $\alpha$ 's and  $\beta$ 's lead to receptors with unique pharmacological properties. Of the various  $\alpha$  transcripts that yield functional receptors not blocked by alpha bungarotoxin,  $\alpha 4$  is the most abundant in the CNS, whereas,  $\alpha 3$  is the most abundant in autonomic ganglia. Structurally, the amino acid composition of  $\alpha 3$  and  $\alpha 4$  subunits are similar, but they differ significantly in the main extracellular domain before the first membrane spanning region (M1) and in the putative intracellular domain located between M3 and M4. In this study, we have compared  $\alpha 3/\beta 2$  receptors and  $\alpha 4/\beta 2$  receptors expressed in oocytes at the single channel level using both outside-out patches and cell-attached patches.  $\alpha 3$  and  $\alpha 4$  have identical amino acid residues across the M2 domain and our preliminary results indicate that  $\alpha 3/\beta 2$  and  $\alpha 4/\beta 2$  receptors have similar single channel conductances when measured in outside-out patches. These results are consistent with the idea that the M2 domain is the channel pore. We have attempted to measure the calcium conductance of these receptors by replacing extracellular sodium for calcium; under these conditions, we have observed very few openings for both  $\alpha 3/\beta 2$  and  $\alpha 4/\beta 2$ ; one possibility is that the calcium currents are too small to resolve.  $\alpha 4/\beta 2$  receptors measured in cell-attached configurations appear to have larger conductances than when measured in outside-out patches, suggesting that cytoplasmic factors may influence the function of these receptors. In outside-out patches, both  $\alpha 3/\beta 2$  and  $\alpha 4/\beta 2$  receptors rundown (a process distinct from receptor desensitization) but appear to do so with different kinetics; we are currently in the process of quantifying this phenomenon. (Supported by the MRC of Canada.)

### SESSION 3: MOLECULAR ASPECTS OF RECEPTORS

#### POSTER ABSTRACTS

#### P12 EVIDENCE FOR FUNCTIONAL DIVERSITY OF NEURONAL nAChRs REGULATING NEUROTRANSMITTER RELEASE IN RAT BRAIN

Evelyn D. Cadman and Stephen P. Arneric, Neuroscience Area, Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, Illinois 60064-3500

Molecular biological techniques have elucidated the existence of multiple subtypes of neuronal nicotinic acetylcholine receptors (nAChR). Distinct functions have yet to be assigned to these various nAChR subtypes *in situ*. The present study was undertaken to see if neuronal nAChR subtypes could be differentiated through functional biochemical studies. The ability of various nicotinic agonists to mimic or antagonists to inhibit nicotinic facilitation of spontaneous neurotransmitter release from slices was compared for dopamine (DA) release from rat striatum and norepinephrine (NE) release from rat cerebral cortex. Identical relative potencies were observed for nicotinic agonists; (-)nicotine  $\geq$  (-)cytisine  $>$  (+)nicotine  $\gg$  lobeline for both DA and NE; although the EC50s differed [(-)Nic EC50: DA = 42nM, NE = 3.4 $\mu$ M]. In contrast, different profiles were observed when various nicotinic antagonists were tested for their ability to reverse the facilitation of spontaneous neurotransmitter release by (-)nicotine. In particular, the neuronal nicotinic antagonist, dihydro- $\beta$ -erythroidine displayed an IC50 of 50nM in the assay of DA release from striatal slices and 9 $\mu$ M in the assay of NE release from cortical slices. Methyllcaconitine inhibited nicotinic facilitation of DA release from striatum (IC50 = 450nM) but failed to fully inhibit nicotinic facilitated release of NE from cortical tissue. These results suggest that differing nAChR subtypes are involved in these phenomenon.

#### P13 CENTRAL NICOTINIC RECEPTOR BLOCKADE NEITHER BLOCKS NOR MIMICS CHRONIC NICOTINE-INDUCED $^3$ H-NICOTINE BINDING UPREGULATION IN RAT BRAIN. H. El-Bizri and P.B.S. Clarke. Dept of Pharmacology and Therapeutics, McGill Univ., Montreal, Canada H3G 1Y6.

Chronic *in vivo* administration of nicotine to rats results in an increased density of  $^3$ H-nicotine binding in the brain. An analogous finding has been reported in humans:  $^3$ H-nicotine binding sites are more numerous in postmortem brain from smokers than from non-smokers. On the face of it, this upregulation of binding appears paradoxical since nicotine is usually regarded as an agonist. To account for this paradox, it has been proposed that the upregulation of  $^3$ H-nicotine binding induced by chronic *in vivo* nicotine reflects long term receptor blockade (through desensitization) rather than stimulation. If this were the case, chronic nicotinic receptor blockade produced by a nicotinic antagonist should mimic but not block the upregulation produced by chronic nicotine treatment. Published studies examining this question are difficult to interpret because the presence of chronic receptor blockade was not verified. In the present study, this issue was reexamined using chlorisondamine (CHL), a nicotinic antagonist which blocks nicotine's CNS effects for many weeks after a single administration. Care was taken to verify the continued presence of central nicotinic blockade over the course of the experiment. We tested whether chronic CNS nicotinic blockade by CHL alters  $^3$ H-nicotine receptor binding or alters chronic nicotine-induced upregulation. Rats were randomly allocated to 4 groups (n=12) and were pretreated with CHL (10 mg/kg sc) or saline, and treated chronically with nicotine (0.6 mg/kg sc bid for 10 days) or saline. Persistent central blockade was assessed in tests of nicotine-induced locomotion given just before and after chronic treatment. These tests showed that CHL did indeed exert a continuous central nicotinic blockade. Moreover, this blockade was not reversed by chronic nicotine treatment. Chronic treatment with nicotine resulted in an increase in the density ( $B_{max}$ ) but not in the affinity ( $K_D$ ) of forebrain  $^3$ H-nicotine binding (19% increase,  $p < 0.001$ ). CHL neither altered  $^3$ H-nicotine receptor binding density when given alone nor did it alter upregulation induced by nicotine. These findings suggest that the signal for  $^3$ H-nicotine binding site upregulation is neither receptor stimulation nor receptor blockade. Supported by NIDA.

## SESSION 3:

## MOLECULAR ASPECTS OF RECEPTORS

## POSTER ABSTRACTS

- P14 THE REMARKABLY LONG TERM BLOCKADE OF CNS NICOTINIC RECEPTORS PRODUCED BY IN VIVO ADMINISTRATION OF CHLORISONDAMINE PERSISTS IN VITRO. H. El-Bizri and P.B.S. Clarke. Dept of Pharmacology and Therapeutics, McGill Univ., Montreal, Canada H3G 1Y6.

Chlorisondamine (CHL) is a bisquaternary ganglion blocking drug which, when administered centrally or in a sufficiently high dose systemically, blocks CNS nicotinic responses in a quasi-irreversible fashion. This blockade occurs after a single administration of the drug, persists for several weeks with no clear sign of recovery, and is pharmacologically selective. The blockade was first noticed in tests of nicotine-induced locomotor activity, but has now been observed in a wide range of behavioural tests which all reflect direct central actions of nicotine. It is likely, therefore, that chlorisondamine's long-acting block is produced at the level of nicotinic receptors. To investigate the mechanism of this blockade, we investigated nicotine-induced  $^3\text{H}$ -dopamine release from rat brain striatal synaptosomes. This is a convenient assay of CNS nicotinic receptor function that has been used quite widely. The first experiments characterized the effects of nicotinic agents given *in vitro*. Synaptosomes were prepared from the crude P2 pellet, incubated with  $^3\text{H}$ -DA, and superfused; 1 min samples were collected. Following 25 min wash, a brief pulse of nicotine and then of high  $\text{K}^+$  buffer were given 10 min apart. Nicotine (0.01 – 100  $\mu\text{M}$ ) induced  $^3\text{H}$ -DA release in a concentration dependent and  $\text{Ca}^{++}$  dependent manner. Release evoked by nicotine (1  $\mu\text{M}$ ) was blocked in a graded fashion by *in vitro* administration of mecamylamine, dihydro-beta-erythroidine and CHL (0.01 – 100  $\mu\text{M}$ ); blockade by CHL was not surmounted by a high concentration of nicotine (100  $\mu\text{M}$ ). In subsequent experiments, CHL (or saline) was given *in vivo* and rats were permitted to survive for varying periods. CHL (10 mg/kg sc), administered *in vivo* at 1, 7, 21 or 42 days before sacrifice, completely blocked  $^3\text{H}$ -dopamine release induced by a 1  $\mu\text{M}$  nicotine challenge administered *in vitro*. These results suggest that chlorisondamine's persistent *in vivo* blockade does not solely result from retention of this bisquaternary amine by the blood-brain barrier. Funded by NIDA.

- P15 PHOTOACTIVATABLE AGONIST OF THE NICOTINIC ACETYLCHOLINE RECEPTOR: POTENTIAL PROBE TO CHARACTERIZE THE STRUCTURAL TRANSITIONS OF THE ACETYLCHOLINE BINDING SITE IN DIFFERENT STATES OF THE RECEPTOR

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The nicotinic acetylcholine receptor exhibits several affinity states for agonists such as acetylcholine. In order to identify structural changes occurring at or near the agonist binding site during the allosteric transitions, photoactivatable compounds designed to display agonist activity were synthesized and tested accordingly by electrophysiological experiments. The AC<sub>5</sub> molecule, a photoactivatable analogue of the fluorescent Dansyl-C5-choline molecule, showed the most promising properties; it is an agonist with high affinity and selectivity and giving high photolabeling yield. The [ $^3\text{H}$ ]AC<sub>5</sub> molecule was shown to label all four receptor subunits in a protectable manner and thus it appears suitable for investigation of the dynamics of allosteric transitions occurring at the activated acetylcholine binding site.

**SESSION 3: MOLECULAR ASPECTS OF RECEPTORS**

**POSTER ABSTRACTS**

**P16 DDF, A PHOTSENSITIVE ANALOGUE OF ACETYLCHOLINE, AS TOPOGRAPHICAL PROBE OF THE AMMONIUM BINDING SITE OF ACETYLCHOLINE**

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DDF is a positively charged aromatic diazonium salt which mimics the quaternary ammonium of acetylcholine. It was possible to establish this chemical analogy both on acetylcholinesterase for which DDF is a competitive inhibitor and on the nicotinic acetylcholine receptor where DDF was demonstrated to be an acetylcholine antagonist. The photoactivation of DDF generates a highly reactive intermediate which is able to react instantaneously with any amino acid residue including the non reactive hydrophobic residues. It has been taken advantage of this property to label irreversibly the acetylcholine binding site on both *Torpedo marmorata* acetylcholinesterase and the nicotinic receptor by using a tritiated DDF probe. Cleavage of the labeled polypeptides, purification of the labeled peptide fragments and finally microsequencing of the radiolabeled fragments allowed an identification of the amino acid residues which were alkylated by this probe. The overall labeling results will be presented on *Torpedo* acetylcholinesterase especially with regard to recent crystallographic data as well as on the nicotinic acetylcholine receptor.



## SESSION 4:

## MOLECULAR BIOLOGY OF CHOLINESTERASES

SYMPOSIUM ABSTRACTS

- S13 **MOLECULAR BIOLOGY OF CHOLINESTERASES.** Heilbronn E. Dept of Neurochemistry and Neurotoxicology, Stockholm University, S-106 91 Stockholm, Sweden.

Enzymes hydrolyzing choline esters, i.e. acetylcholinesterases (AChE), splitting preferentially acetylcholine (ACh), and cholinesterases (ChE) have been studied *in vivo* and *in vitro* since the 1920s, when Dale and then Loewi demonstrated the importance of ACh and its rapid removal in cholinergic neurotransmission. Animal and tissue specificity, biochemistry and pharmacology, developmental aspects, role in ageing and in disease, toxicological aspects, usefulness as a clinical and environmental analytical (biomonitor) tool have been thoroughly studied. Probing into the structure of various types of AChE and ChE started with structure-activity studies using substrates and inhibitors, structural differences between AChE and ChE became obvious. Simultaneously, enzyme purification was tried by traditional biochemical techniques. The use of radioactively labelled organophosphate inhibitors resulted in first data on short amino acid sequences from the ChE phosphorylation site; today the primary structures of several ChEs are known. Both AChE and ChE exist in several molecular forms, assembled from several catalytic or catalytic and other (asymmetric forms) subunits. Their modes of membrane attachment are known. Their primary structure, showing a relation to other secretory proteins, points to proteins designed for secretion from the cell and recently, stimulation-related release of ChE from neurons was observed but its function is not understood. Current work aims at a complete functional mapping of the enzymes and at an understanding of the existence of the many diverse molecular species of AChE and ChE and of their relation to physiology and pathology.

- S14 **EXPRESSION OF TORPEDO AND RAT ACETYLCHOLINESTERASE FORMS IN TRANSFECTED CELLS**

Jean Massoulié, Suzanne Bon, Nathalie Duval, Claire Legay, Eric Krejci and Françoise Coussen

*Laboratoire de Neurobiologie, Ecole Normale Supérieure, 46 rue d'Ulm, 75005 Paris, France*

The molecular forms of cholinesterases differ in their quaternary oligomeric structure and their modes of anchoring: they may be attached to basal laminae (asymmetric collagen-tailed forms) or to plasma membranes (amphiphilic forms). Two types of catalytic subunits (H and T), generated by alternative splicing of the mRNA transcripts, possess distinct C-terminal peptides. We expressed H and T subunits of AChE in transfected COS cells. *Torpedo* H subunits produce a single form, glycolipid-anchored dimers (GPI-G<sub>2</sub><sup>a</sup>), as *in vivo*. In contrast, both *Torpedo* and rat T subunits produce a variety of forms. These include non-amphiphilic tetramers (G<sub>4</sub><sup>na</sup>) and a large proportion of amphiphilic dimers and monomers (G<sub>2</sub><sup>a</sup> and G<sub>1</sub><sup>a</sup>), similar to amphiphilic forms of type II, which are abundant in the nervous tissue and muscles of higher vertebrates. The amphiphilic nature of these molecules is due to the presence of the C-terminal peptide T, since truncated subunits generated only non-amphiphilic monomers. When co-expressed in COS cells, the collagenic subunit of *Torpedo* asymmetric AChE combines with *Torpedo* T subunits, and also with rat T subunits. This demonstrates: a) that the formation of these complex forms does not require a specific biosynthetic capacity of differentiated cells, and b) that the complementarity of the catalytic T subunit and collagenic subunit is very well conserved between vertebrates.

## SESSION 4: MOLECULAR BIOLOGY OF CHOLINESTERASES

### SYMPOSIUM ABSTRACTS

- S15 **Human Cholinesterase Genes: Molecular Dissection and Biomedical Implications**  
Hermona Soreq, Department of Biological Chemistry, Institute of Life Sciences, The Hebrew University of Jerusalem, 91904, Israel.
- The acetylcholine hydrolysing enzymes cholinesterases (CHEs) control the termination of intercellular communication in brain, muscle and multiple embryonic tissues. In addition, they serve as the natural scavengers for a variety of synthetic and natural drugs. The two human genes encoding acetyl- and butyrylcholinesterase (ACHE, BCHE) were isolated by genetic engineering techniques and their chromosomal locations were mapped to 7q22 and 3q26-ter, respectively. The cloned genes were used to demonstrate expression patterns of ACHE and BCHE in human brain, germ cells and fetal chorionic villi, suggesting that environmental exposure to CHE inhibitors subjects the ACHE and BCHE genes to evolutionary selection pressure. Several different point mutations in CHE genes were found to be abundant in the Israeli population. Analytical expression studies of these and other CHE mutants in microinjected oocytes of the frog *Xenopus laevis* have proven that certain allelic variants of CHE genes conferred resistance to several CHE inhibitors, which may explain the evolutionary emergence of these multiple alleles. Moreover, abnormal transcription and *in vivo* amplification of the ACHE and BCHE genes has been variously associated with abnormal production of blood platelets, leukemias and brain and ovarian tumors. More recently, "antisense" oligonucleotides blocking the expression of CHE genes were shown to interfere with bone marrow development, implicating these genes in influencing cell growth and proliferation. We are currently examining the possibility that mutations in the CHE genes are causally involved in fertility and tumorigenesis and that exposure to anti-CHE organophosphorous poisons, creating ecological pressures on the human genetic repertoire, alters the incidence of such mutations.
- S16 **THE CHOLINESTERASES: SOME UNANSWERED QUESTIONS** V.P. Whittaker, Arbeitsgruppe Neurochemie des Anatomischen Instituts der Johannes Gutenberg-Universität Mainz, FRG
- As 'Discussant' in the session on the molecular biology of cholinesterases I shall briefly review some of the earlier results on the specificity and kinetics of these enzymes which the newer information on their amino-acid sequences and tertiary structure must accommodate, and shall then pass on to pose some unanswered questions which future research should attempt to clarify. Among these are the following.
- How does the newer information account for the differences between acetyl- and butyrylcholinesterase in substrate and inhibitor specificity and kinetics?
  - What proportion of synaptic acetylcholinesterase is contributed by pre- and post-synaptic components of a cholinergic synapse? Does this vary at different synapses? If so, why?
  - What is the functional significance of the polymeric forms of acetylcholinesterase and how are they assembled?
  - What is the significance and function of the considerable proportion of both types of cholinesterase found in nonsynaptic locations.

## SESSION 4: MOLECULAR BIOLOGY OF CHOLINESTERASES

### POSTER ABSTRACTS

#### P17 STRUCTURAL AND FUNCTIONAL OF *TORPEDO* ACETYLCHOLINESTERASE: THREE-DIMENSIONAL STRUCTURE AND SITE-DIRECTED MUTAGENESIS

I. Silman<sup>1,3</sup>, M. Harel<sup>2</sup>, E. Kreici<sup>3</sup>, N. Duval<sup>3</sup>, S. Bon<sup>3</sup>, P. Chanal<sup>3</sup>, J. Massoulié<sup>3</sup> & J.L. Sussman<sup>2</sup>

*Depts of Neurobiology*<sup>1</sup> & *Structural Biology*<sup>2</sup>, *The Weizmann Institute of Science, Rehovot 76100, ISRAEL* and

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The main role of acetylcholinesterase (AChE) is termination of impulse transmission at cholinergic synapses by rapid hydrolysis of acetylcholine (ACh). Based on our recent X-ray crystallographic structure determination of *Torpedo californica* AChE (Sussman *et al. Science* 253, 872-879, 1991), it was possible to visualize, for the first time, at atomic resolution, a protein-binding pocket for ACh. It was found that the active site consists of a catalytic triad (S200-H440-E327) located near the bottom of a deep and narrow gorge lined with the rings of 14 aromatic amino acid residues. Despite the complexity of this array of aromatic rings, we proposed, on the basis of modelling which involved docking of ACh in an all-*trans* conformation, that the quaternary group of the choline moiety makes close contact with the indole ring of W84. A variety of AChE inhibitors have been synthesized and characterized pharmacologically, since symptomatic treatment of various diseases can be achieved by controlled inhibition of AChE. Inhibition of AChE with anticholinesterase agents is thus of therapeutic importance for countering such diseases as glaucoma and myasthenia gravis, and in the possible management of Alzheimer's disease. To study the interactions of AChE with these agents, we have soaked into crystals of AChE a series of inhibitors, and recently determined the 3-D structure of complexes of AChE with two anticholinesterase drugs, edrophonium and tacrine. Edrophonium is a quaternary ammonium compound which acts peripherally, and is used in the diagnosis of myasthenia gravis. Tacrine is a tertiary analog of acridine which penetrates the blood-brain barrier and is currently being evaluated in the management of Alzheimer's disease. The crystal structures of the two complexes are in good agreement with our model building of ACh bound in the active site of AChE, and show that the interaction of both drugs with AChE produces localized conformational changes whose significance we are currently evaluating. Site-directed mutagenesis is being performed on selected amino acids within the active-site gorge, to gain an understanding of the functional significance of this unusual structure. This is being achieved by manipulation of the *T. marmorata* AChE gene by conventional techniques, followed by transfection into COS cells. Among the residues being mutated are some of the highly conserved aromatic amino acids of the gorge, as well as several acidic residues of possible structural, as well as functional, significance. Results of the mutagenesis experiments will be evaluated and discussed in relation to the three-dimensional structure of AChE.

#### P18 EVALUATION OF GENE EXPRESSION LEVELS IN CHOLINERGIC SIGNALLING PATHWAYS BY RNA-PCR AMPLIFICATION

Dalia Ginzberg<sup>1</sup>, Gal Ehrlich<sup>1</sup>, Rachel Beeri<sup>1</sup>, Rachel Karpel<sup>1</sup>,  
Haim Zakut<sup>2</sup> and Hermona Soreq<sup>1</sup>

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Proteins involved in cholinergic signalling pathways are generally expressed in very low levels (i.e., acetylcholinesterase represents ca. 0.001% of total brain protein) and are controlled by intricately coordinated mechanisms ("cross-talk"). To decipher the correlation between expression levels of the corresponding genes, a sensitive, selective method is required for the detection and quantification of their mRNA products. For this purpose, we have initiated the use of direct polymerase chain reaction (PCR) for amplification of cDNA fragments reverse-transcribed from RNA extracted from minute samples of tissue or cultured cells. RNA-PCR amplification was performed for the mammalian acetyl- and butyrylcholinesterase (ACHE, BCHE) genes as well as for the cell division controller (cdc) genes 2Hs and CHED and for actin as a housekeeping control gene. The systems employed included brain tissue from several dissected regions and developing bone marrow cells from semi-solid and liquid cultures. For calibration, several plasmids were constructed with internal deletions and their synthetic mRNA products employed as internal standards. Our findings demonstrate variabilities in the RNA-PCR efficiencies which depend on the nucleotide composition of the employed sequences, on their concentration and on their ability to compete on the PCR primers with other cDNA sequences in the complex mixtures employed. Thus, detailed calibration curves need to be developed for each of the studied genes within its natural milieu.

## SESSION 4:

## MOLECULAR BIOLOGY OF CHOLINESTERASES

## POSTER ABSTRACTS

- P19 IN FREELY EXERCISING RATS, ACETYLCHOLINESTERASE ADAPTS BY A MASSIVE  $G_4$  INCREASE IN HINDLIMB FAST MUSCLES TOGETHER WITH AN  $A_{12}$  DECLINE IN SOLEUS MUSCLE. V. Gisiger, M. Bélisle and P.F. Gardiner. Départements d'Anatomie et d'Education Physique, Université de Montréal, Montréal, Québec, Canada, H3C 3J7.

The bulk of  $A_{12}$ , one of the 6 AChE molecular forms, is concentrated at the neuromuscular junction where it is responsible for the rapid hydrolysis of ACh. However, recent evidence indicates that the tetrameric  $G_4$  form, another major muscle AChE component, occupies mainly perijunctional sites and is specifically related to muscle dynamic state. In particular, chronic enhancement of neuromuscular activity by exercise results in a selective  $G_4$  adaptation in fast muscles of the rat, contrasting with only small, non-specific changes in slow muscles. In order to ascertain the physiological significance of the selective  $G_4$  adaptation obtained by coercive training, we studied the impact of voluntary activity on muscle AChE. Rats were placed in wheel cages in which they spontaneously ran for distances which, while varying widely among individual animals, progressively increased from about 0.5-10 km/day, at the 5th day, to about 2-20km/day at the end of the 4th week. The effect of this voluntary activity on AChE was examined in fast hindlimb muscles as well as soleus muscle. All 3 fast muscles examined exhibited a massive selective increase in  $G_4$ , with only minor changes in  $A_{12}$ , so that the tetramer became largely predominant by the 4th week. Remarkably, the  $G_4$  content of fast muscles, which varied extensively among individual animals, exhibited a tight correlation with the distance covered by each rat. In opposition to its marginal response to training, the soleus muscle adapted to wheel cage activity by a marked selective reduction of its asymmetric forms, up to 45 % in the case of  $A_{12}$ . Interestingly, the  $A_{12}$  decline was already maximal by the 5th day and showed no relationship with the distance covered. These adaptations appear difficult to reconcile with the classical concept limiting the role of muscle AChE to the rapid inactivation of just-released ACh. The present results strongly support the proposal (J. Neurosci. 10, 1444, 1990) that muscle AChE controls additional functional parameters, including endplate excitability. With the support of FCAR.

- P20 ACETYLCHOLINESTERASE (AChE) AND BUTYRYLCHOLINESTERASE (BChE) ACTIVITY IN HUMAN BRONCHIAL PREPARATIONS (HB).  
NOREL, X., <sup>a</sup>ANGRISANI, M., LABAT, C., GORENNE, I., <sup>a</sup>ROSSI, F. & BRINK, C.  
CNRS URA 1159, HOPITAL MARIE-LANNELONGUE, 133 Av. de la Résistance, 92350 LE PLESSIS-ROBINSON, FRANCE. <sup>a</sup> ISTITUTO DI FARMACOLOGIA E TOSSICOLOGIA, Via Costantinopoli, 16 - 80138 NAPOLI, ITALIA.

Biochemical experiments have shown that acetylcholine (ACh) is degraded by both AChE and BChE while butyrylcholine (BuCh) is mainly hydrolyzed by BChE. These enzymatic degradations may be prevented by specific inhibitors (iso-OMPA and neostigmine). HB rings were placed in 5 ml Tyrode's solution (TSS) gassed with 5%  $CO_2$  in 95%  $O_2$  at 37°C. After 1 hour equilibration, the preparations were incubated in TSS with or without cholinesterase inhibitors for 30 min prior to challenge with ACh or BuCh (0.5  $\mu$ moles) and during the 3 hours period following substrate treatment. Detection of cholinergic agonists ( $\mu$ moles) remaining in supernatants 3 hours after challenge were determined using a chemiluminescence technique and are presented in table as means  $\pm$  SEM.

	Control	iso-OMPA 10 $\mu$ M	neostigmine		iso-OMPA 10 $\mu$ M and neostigmine 0.01 $\mu$ M
			0.01 $\mu$ M	10 $\mu$ M	
ACh	0.10 $\pm$ 0.07 (7)	0.21 $\pm$ 0.08 (4)	0.20 $\pm$ 0.08 (4)	0.49 $\pm$ 0.05 (5)	0.31 $\pm$ 0.09* (4)
BuCh	0.05 $\pm$ 0.03 (4)	0.48 $\pm$ 0.04* (4)	0.08 $\pm$ 0.04 (3)	0.26 $\pm$ 0.03 (3)	ND

In parentheses: number of lung samples used. \* result significantly different from values obtained with an inhibitor alone or control.

These results suggest a co-regulation of ACh degradation by BChE and AChE in HB.

## SESSION 5: SYNTHESIS AND STORAGE OF ACh

### SYMPOSIUM ABSTRACTS

- S17 **SYNTHESIS AND STORAGE OF ACETYLCHOLINE.** R. Jane Rylett. Department of Physiology, University of Western Ontario, London, Ontario, Canada.

Cellular and molecular mechanisms regulating the synthesis, storage and release of ACh will be considered in this Session. Synthesis of the neurotransmitter can be controlled by the provision of the precursors choline or acetylCoenzyme A, or at the level of the biosynthetic enzyme choline acetyltransferase. Accumulation of choline into the presynaptic terminal by the sodium-coupled, high-affinity transport system has long been considered the rate-limiting step for ACh biosynthesis. Although functional regulation of choline carrier activity has not been elucidated, recent evidence indicates that the number of transporters or the rate of transport may be controlled at least in part by some intracellular second messenger systems. Provision of the co-substrate acetylCoenzyme A does not appear to represent a rate-controlling step in ACh synthesis under normal conditions, but may limit production of the transmitter under some conditions including hypoxia and hypoglycemia or during aging. It has generally been thought that ChAT does not play a regulatory role in ACh biosynthesis as it is present in the neuron in kinetic excess. More recently, attention has focussed upon phosphorylation and subcellular localization of the enzyme in cytosolic and membrane compartments as possible mechanisms for regulating enzyme catalytic activity and function. The amino acid sequence of the enzyme elucidated from the cloned nucleotide sequence has revealed potential phosphorylation sites on the protein, but the biological significance of this remains to be determined. Similarly, the existence of different forms of the enzyme associated with different subcellular compartments is still controversial. In terms of function, the role of membrane-associated ChAT in ACh synthesis during repetitive neuronal activity must be tested, but it appears that this form of the enzyme does not regulate production of the transmitter under basal conditions.

- S18 **MOLECULAR GENETIC SPECIFICATION OF CHOLINERGIC NEURONS** PAUL SALVATERRA, TOSHIHIRO KITAMOTO AND KAZUO IKEDA BECKMAN RESEARCH INSTITUTE OF THE CITY OF HOPE, 1450 E. DUARTE RD. DUARTE, CA, 91010, USA

Cholinergic neurons express choline acetyltransferase (ChAT) which gives them the ability to make and use acetylcholine as a neurotransmitter. ChAT expression is regulated in part at the gene transcriptional level and we are determining the cis-DNA regulatory elements which control this. We have been using *Drosophila* as a model system to study ChAT regulation since it is possible to investigate many aspects of spatial and temporal control by making transgenic animals. DNA fragments from the 5' flanking region of the *Drosophila* ChAT gene were fused to the *E. coli lacZ* reporter gene and introduced into the *Drosophila* germ line by P-element-mediated transformation. A 7.4 kb 5'-flanking sequence directed *lacZ* expression in the adult optic lobes and other well defined CNS structures with a pattern very similar to the distribution of endogenous ChAT protein. This DNA fragment thus contains most, if not all, of the cis regulatory elements required for the correct spatial expression of ChAT. In contrast, pieces of the proximal 5' flanking DNA direct *lacZ* expression in only selected subsets of cholinergic neurons. We also see subset specific expression patterns when *lacZ* is analyzed in embryos or other developmental stages. A number of mutant alleles are available for the *Drosophila* ChAT gene (*Cha*). We have constructed transgenic flies with the different cis-regulatory DNA fused to a wild type ChAT cDNA, and placed these transgenes into animals with a mutant *Cha* background. It is thus possible to supply functional ChAT to various subsets of cholinergic neurons and analyze the mutant/transgenic animals for phenotypic rescue. Most of the 5' flanking DNA sequences can rescue a temperature sensitive allele (*Cha<sup>ts1</sup>*) from adult or embryonic lethality. We can also rescue flies for a number of visual or motor phenotypes including adult paralysis and optomotor response. Interestingly, transgenic flies have a very strong correlation between total motor activity and the absolute amount of transgenic (i.e. wild type) ChAT enzyme being expressed in the animal.

**SESSION 5:           SYNTHESIS AND STORAGE OF ACh**

**SYMPOSIUM ABSTRACTS**

**S19 ACETYLCHOLINE TRANSPORTER-VESAMICOL RECEPTOR PHARMACOLOGY AND STRUCTURE.**

**S.M. Parsons, B.A. Bahr, G.A. Rogers, E.D. Clarkson, K. Noremborg, and B.W. Hicks.**  
Department of Chemistry, University of California, Santa Barbara, CA. 93106 USA

The relationship of the acetylcholine (ACh) and vesamicol binding sites to each other in synaptic vesicles isolated from the electric organ of Torpedo was characterized by using analogues of ACh and vesamicol possessing various favorable characteristics. ACh analogues with phenyl substituents on either the acetyl or ammonium ends of the molecule and with 5- or 6-membered rings connecting the carbonyl and ammonium pharmacophores are actively transported. Active transport of ACh and its analogues is inhibited with positive cooperativity in a noncompetitive manner by a low affinity analogue of vesamicol, methoxyvesamicol. Equilibrium binding of [<sup>3</sup>H]vesamicol is inhibited by ACh, and this could be shown to be competitive inhibition in the case of a high affinity analogue of ACh. A kinetics model is proposed in which vesamicol binds to an allosteric site in the ACh transporter (AChT), which exhibits very low affinity and specificity for ACh. The vesamicol receptor (VR) was purified in cholate-solubilized form using the binding of [<sup>3</sup>H]vesamicol as the assay. It is a proteoglycan, and both the SV1 and SV2 epitopes copurify. The AChT was photoaffinity labeled with a tritiated high affinity analogue of ACh. The same proteoglycan was identified by SDS PAGE and autofluorography. The VR in vesicles permeabilized with cholate was inactivated by incubation with keratanase and testicular hyaluronidase, which degrade certain glycosaminoglycan components of proteoglycans. We conclude that the AChT-VR of synaptic vesicles is tightly linked to a proteoglycan. It exhibits active transport kinetics consistent with a channel-like structure that must contain at least two gates in order to account for energy input and prevent leakage of protons.

## SESSION 5: SYNTHESIS AND STORAGE OF ACH

### POSTER ABSTRACTS

- P21 THE PERFUSED ELECTRIC ORGAN AS A TOOL IN CHOLINERGIC PHARMACOLOGY V.P. Whittaker, Arbeitsgruppe Neurochemie des Anatomischen Instituts der Johannes Gutenberg-Universität Mainz, FRG

The electric organs of *Torpedo marmorata* are removed together with lengths of the electromotor nerves and with the ventral and dorsal skin still adhering, divided along the line between the territories of the IIInd and IIIrd nerves and perfused through fine nylon cannulae inserted into the tissue blocks along the tracks of the blood-vessels which accompany each nerve, using a roller pump at a flow rate of 2 ml per min. Perfusion may be open or closed; labelled compounds or drugs may be added to the perfusate and closed-circuit perfusion may be interrupted to wash out label or drugs or to collect perfusate, e.g. during a period of nerve stimulation. A variety of pharmacological experiments may be done on such blocks; at the end of the experiment synaptosomes may be prepared from them or they may be frozen and synaptic vesicles isolated in the usual way. Using this preparation we have recently shown that vesamicol not only blocks the uptake of recently synthesized acetylcholine into recycling vesicles, but also halts the recovery by such vesicles of the biophysical properties of reserve vesicles during an ensuing period of rest. This supports the theory that these biophysical changes are a function of the vesicles' osmotic load and degree of hydration (1,2). We have also used the preparation to study muscarinic receptors and the release of the cholinergic cotransmitter VIP from electromotor terminals (3).

1. Giompres PE & Whittaker VP (1986) BBA 882: 398-409
2. Agoston DV et al (1986) J Neurochem 47: 1584-1592
3. Whittaker VP (1992) The Cholinergic Neuron and its Target: The Electromotor Innervation of the Electric Ray *Torpedo* as a Model. Birkhäuser, Boston

- P22 PHARMACOLOGICAL CHARACTERIZATION OF A SERIES OF NOVEL ANALOGS OF HEMICHOLINIUM-3 K.H. Gyls, I. Abdalla, M. Roch, K.M. Rice, D.J. Jenden, Shen Wei and U. Hacksell. UCLA School of Medicine, Los Angeles, California, USA & Dept of Organic Pharmaceutical Chemistry, Uppsala University, Uppsala, Sweden.

Six new compounds comprising three bisquaternary ammonium compounds and their N-desmethyl analogs were prepared and examined for inhibitory activity against high affinity choline uptake in rat brain synaptosomes (HACU), acetylcholinesterase (AChE; *E. electricus*), choline acetyltransferase (ChAT; from rat striatum), and displacement of N-methylscopolamine from rat brain membrane preparations. The base compound (II, 4,4'-bis[N,N'-dimethyl-3,3'-pyridinyl]-biphenyl) was most potent for HACU inhibition. In the remaining quaternary compounds, the pyridine ring was partially (IV) or completely (VI) reduced. Compounds I, III & V were N-desmethyl analogs of II, IV & VI respectively. The IC<sub>50</sub> concentrations (nM) were as follows:

Compound	HACU	AChE	NMS	CAT
HC3	20	68,600	-	> 100,000
I	> 100,000	14,200	> 100,000	> 100,000
II	5	51	5,220	> 100,000
III	79	973	4,020	> 100,000
IV	37	390	8,350	> 100,000
V	312	3,620	11,590	> 100,000
VI	51	3,290	24,150	> 100,000

These compounds provide new information with respect to the HACU structure-activity relationship, and may be useful probes for this transport system. (Supported by USPHS grant MH 17691)

## SESSION 5: SYNTHESIS AND STORAGE OF ACH

### POSTER ABSTRACTS

**P23 STERESELECTIVITY OF THE INHIBITION OF [<sup>3</sup>H]HEMICHOLINIUM-3 BINDING TO THE SODIUM-DEPENDENT HIGH-AFFINITY CHOLINE TRANSPORTER IN RAT STRIATUM. S. S. G. Ferguson and B. Collier. Department of Pharmacology and Therapeutics, McGill University, Montreal, Canada H3G 1Y6.**

High-affinity choline transport shows an absolute stereospecificity for translocation of the enantiomers of  $\alpha$ - and  $\beta$ -methylcholine (MeCh), whereas it recognizes these chiral compounds with a relative stereoselectivity. The inhibition of [<sup>3</sup>H]Hemicholinium-3 (HC-3) binding by the enantiomers  $\alpha$ - and  $\beta$ -MeCh has been examined in order to investigate whether inhibition of choline transport by HC-3 is the result of its interaction with either the transport or the recognition component of choline transporter. S(+)- $\beta$ -MeCh was five times more effective than its enantiomer at inhibiting the binding of [<sup>3</sup>H]HC-3 to crude striatal membranes, while, R(+)- $\alpha$ -MeCh, although eight times more effective than its enantiomer at inhibiting striatal high-affinity choline transport, was only twice as effective an inhibitor of [<sup>3</sup>H]HC-3 binding. However, S(+)- $\beta$ -MeCh and R(+)- $\alpha$ -MeCh inhibited [<sup>3</sup>H]HC-3 binding to striatal synaptosomes 5 and 6.5 times better than their enantiomers. Examination of the affinity of [<sup>3</sup>H]HC-3 for its binding site in these two preparations revealed that [<sup>3</sup>H]HC-3 binds with both high- and low-affinities to crude striatal membranes, but with only low-affinity to striatal synaptosomes. The addition of 1 mM Ca<sup>2+</sup> and Mg<sup>2+</sup> as well as 0.5 mM ATP to the crude membrane preparation resulted in a single affinity [<sup>3</sup>H]HC-3 binding sites, with the enantiomers of  $\alpha$ -MeCh now exhibiting stereoselectivity in their ability to inhibit [<sup>3</sup>H]HC-3 binding (eudismic ratio, 4.6). In contrast, when striatal synaptosomes were washed with EDTA, the stereoselective inhibition of [<sup>3</sup>H]HC-3 binding exhibited previously by the enantiomers of  $\alpha$ -MeCh was lost (eudismic ratio, 1.6). In conclusion, low-affinity [<sup>3</sup>H]HC-3 binding was stereoselectively inhibited by the enantiomers of both  $\alpha$ - and  $\beta$ -MeCh, but high-affinity [<sup>3</sup>H]HC-3 binding was stereoselectively inhibited by only the  $\beta$ -MeChs. This would suggest that HC-3 inhibits choline transport via an interaction with the substrate recognition component, but not the transport component of the high-affinity choline transporter, although the physical-chemical properties of the recognition site may differ depending upon the affinity-state of the transporter.

Supported by MRC-Canada and the Alzheimer Society of Canada.

**P24 MOBILIZATION OF A VESAMICOL-INSENSITIVE POOL OF ACETYLCHOLINE BY OUABAIN. <sup>1,2</sup>M.A.M. Prado, <sup>3</sup>M.V. Gomez, <sup>1</sup>B. Collier. <sup>1</sup>Dept. of Pharmacology and Therapeutics, McGill University, 3655, Drummond St., H3G 1Y6, Montreal, PQ Canada and Dept. de Bioquímica e Farmacologia, UFMG, Belo Horizonte, MG, CP 24861-1, Brazil.**

Vesamicol, an inhibitor of acetylcholine (ACh) uptake by synaptic vesicles, has been used to manipulate the intracellular storage of acetylcholine in the cat superior cervical ganglion (SCG). In the presence of this drug, perfused SCG releases just transmitter contained in a readily releasable pool when electrically stimulated (5 Hz) through its preganglionic nerve. However, ouabain releases ACh from the SCG in a Ca<sup>2+</sup>-dependent way and, in the presence of vesamicol, the ATPase inhibitor induced the release of a fraction of transmitter significantly larger than that released by electrical impulses. To test whether ouabain is able to mobilize a vesamicol-insensitive pool of transmitter, which has been described to exist in the SCG (Cabeza and Collier, 1988), ganglia were perfused with vesamicol (10  $\mu$ M) and [<sup>3</sup>H]-choline (754 dpm/pmol, 5  $\mu$ M), stimulated at 5 Hz for 25 min to deplete the readily releasable compartment, and then, after 15 min wash period, ouabain (10  $\mu$ M) was perfused for 60 min in the continued presence of vesamicol. In this protocol, ouabain released an enormous amount of transmitter from the SCG, and this release peaked around 30 min. Acetylcholine synthesized in the presence of vesamicol was also released by the ATPase inhibitor in this condition, and the specific activity of released ACh was always larger than that in the ganglia (peak release S.A. 322 $\pm$ 57, ganglia S.A. 201 $\pm$ 19). Using similar protocol, we tested whether, after treatment with ouabain, the number of synaptic vesicles were decreased in the varicosities. Thus, after the experiment, the SCG were fixed for electron microscopy and micrographs from test and control ganglia compared. The ouabain treated ganglia showed an almost complete depletion in the population of synaptic vesicles. Next, the Ca<sup>2+</sup> dependence of transmitter release by ouabain in this protocol was studied. Therefore, the same experiment was repeated, but after the depletion of the more releasable pool, the SCG were perfused with medium in which Ca<sup>2+</sup> was substituted for Mg<sup>2+</sup> (18 mM) or EGTA (1 mM). In medium that had high Mg<sup>2+</sup>, the SCG treated with ouabain did not release transmitter above basal values, however, in the experiments with EGTA, ouabain caused some release of ACh, but in much smaller amount than that in the presence of Ca<sup>2+</sup> (27% from the experiments done in the presence of Ca<sup>2+</sup>). We conclude that ouabain, in the SCG, is able to release a vesamicol-insensitive population of synaptic vesicles, and Ca<sup>2+</sup> entering in the nerve terminals, probably via the Na<sup>+</sup>-Ca<sup>2+</sup> exchanger, might have a role in the mobilization of this transmitter store.

Supported by MRC-Canada and CNPq-Brazil



## SESSION 5: SYNTHESIS AND STORAGE OF ACh

### POSTER ABSTRACTS

#### P25 INVOLVEMENT OF ADENOSINE AND THE NBTI-RESISTANT NUCLEOSIDE TRANSPORTER IN THE REGULATION OF ACETYLCHOLINE SYNTHESIS AT A SYMPATHETIC GANGLION A. Tandon and B. Collier Dept. of Pharmacology, McGill Univ., Montreal

Exposure of resting cat superior cervical ganglia to adenosine (100 $\mu$ M) increases ACh stores by 25%; this extra ACh mixes with pre-existing stores and can be liberated upon preganglionic stimulation. This action of adenosine appears not to be propagated through extracellular adenosine receptors but through an internal site after uptake through nitrobenzylthioinosine (NBTI)-resistant nucleoside transporters. Moreover, we have tested the role of the NBTI-resistant transporter in this phenomenon using two benzodiazepine stereoisomers, meclonazepam and RO 11-3624 (100 $\mu$ M each). The former does not block the NBTI-resistant transporter at this concentration, whereas the latter does. Adenosine increased ACh stores in the presence of meclonazepam ( $117 \pm 4\%$ ) but not in the presence of RO 11-3624 ( $97 \pm 8\%$ ) supporting the notion that adenosine is internalized through NBTI-resistant transporters prior to its action on ACh synthesis. In addition, other properties of the extra ACh were examined. The stability of the extra ACh was estimated by measuring the ACh content at two time points after the removal of adenosine; ACh stores were augmented by  $25 \pm 5\%$  and by 12% at 15 min. and 75 min., respectively, following adenosine exposure. To test whether the extra ACh affects the size of the readily-releasable pool of ACh, adenosine treated and control ganglia were stimulated (5Hz) in the presence of vesamicol, a drug which inhibits the vesicular ACh transporter. The ACh output from adenosine treated and control ganglia was  $256 \pm 55$  and  $242 \pm 45$  pmoles, respectively. We propose a novel mode of action for adenosine; the nucleoside is transported to an intracellular site to stimulate the synthesis of new ACh which has an approximate half-life of 75 min and incorporates into the less readily-releasable portion of the 'depot' pool. Supported by the MRC of Canada.

#### P26 THE AFFINITY PURIFICATION OF THE ADENOSINE A<sub>2</sub> RECEPTOR

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The rat adenosine A<sub>2a</sub> receptor is localized in the striatum in rodent and human brain, where it stimulates acetylcholine release. With a view to characterize this receptor, it has been solubilized from rat striatal membranes with the zwitterionic detergent CHAPS (1% w/v, 30min at 4°C) with a yield of 40%. The solubilized receptor was assayed by its ability to bind <sup>3</sup>H CGS 21680 using polyethyleneimine soaked filters. The solubilized A<sub>2</sub> receptor retained the same pharmacological profile as the membrane-bound receptor, the order of potency being CGS 21680 > CP66, 713 > R-PIA > DPCPX. An agonist affinity column was then designed for the affinity purification of the receptor. Application of the solubilized receptor (16h, 4°C) resulted in the retention of all the receptor solubilized from 1g of rat striatal tissue. This column therefore constitutes a suitable affinity matrix for the purification and characterization of the mammalian A<sub>2</sub> receptor.

## POSTER ABSTRACTS

## P27 IDENTIFICATION OF A PROMOTER REGION OF THE RAT CHOLINE ACETYLTRANSFERASE GENE

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*Laboratoire de Génétique Moléculaire de la Neurotransmission et des Processus Neurodégénératif, CNRS, Gif-sur-Yvette, France.*

Choline acetyltransferase (ChAT), the enzyme responsible for the synthesis of acetylcholine, provides a convenient index for cholinergic neurons. Using a previously identified rat cDNA clone, we have isolated several corresponding genomic clones. Their analysis reveals the presence of a 7kb intron in the 5' non coding region of the cDNA and a 1,902 bp HindIII fragment that contains part of the first exon. Three transcription start sites were localised at positions -703, -739 and -886 from the translation initiation codon. These sites were determined by the recently developed SLIC strategy, which is based on the ligation of an oligonucleotide to the 3' of single strand cDNAs. The ligation products are then amplified using PCR. No TATA box is found upstream these start sites. However, the sequence surrounding two of them resembles the consensus motif of the Initiator, a *cis*-element that has been shown to promote accurate basal transcription of genes that lack TATA box. The 1902 bp fragment was shown to be transcriptionally active when linked to a CAT reporter gene and transfected into primary cultured cells derived from embryonic septa, a structure in which 1 to 5% of the cells are cholinergic. Moreover, addition of NGF to the culture increases the endogenous ChAT and CAT activities by 2.5 and 56 fold, respectively, indicating that this fragment contains sequences required for NGF induction. Experiments are now in progress to determine whether AP1 sites, present in the promoter, are involved in this induction. The sequence of the promoter contains also consensus sites for other transcription factors, including those of the basic helix-loop-helix family.

P28 TWO HUMAN CHOLINE ACETYLTRANSFERASE mRNAs ARE PRODUCED BY ALTERNATIVE SPLICING. W.L. Strauss, M.V. Lorenzi, and A.C. Trinidad. Dept. of Molecular and Cellular Pharmacology, University of Miami School of Medicine, P.O. Box 016189, Miami, FL 33101 USA.

Choline acetyltransferase (ChAT), the biosynthetic enzyme for acetylcholine, is encoded by a single human gene located on chromosome 10q11-q22.2. A 14.4 kb clone that contains 7 exons of this gene has been used to study human ChAT gene transcription. A restriction fragment from the 5'-end of this clone contains an exon with high identity to the region encoding the amino terminus of porcine ChAT (Berrard *et al.* PNAS 84: 9280, 1987) and detects 2 mRNAs (2300 nt and 6000 nt) on Northern blots of poly A<sup>+</sup> RNA isolated from CHP134 human neuroblastoma cells. Both the 2300 nt and 6000 nt human ChAT gene transcripts also can be detected in total RNA isolated from human nucleus basalis using a ribonuclease protection assay. A restriction fragment from the 3'-end of the genomic ChAT clone contains another protein coding exon, but hybridizes only to the 2300 nt mRNA. cDNA clones corresponding to both the 2300 nt and 6000 nt mRNAs have been isolated. A comparison of these sequences with other mammalian ChAT cDNA sequences suggests that the 2300 nt transcript encodes enzymatically active human ChAT. The predicted translation product from the 2300 nt human ChAT mRNA has 90% and 86% identity with the proteins predicted from the porcine and rat ChAT cDNA clones, respectively (Berrard *et al.* PNAS 84: 9280, 1987; Brice *et al.*, J. Neurosci. Res. 23: 266, 1989). As noted previously (Strauss *et al.* Genomics 2: 396, 1991), however, the initiator MET for porcine and rat ChAT is replaced by THR in human ChAT, so that the translation start site for the latter protein is not known. A comparison of the sequences of the cDNAs derived from the 2300 nt and 6000 nt human ChAT mRNAs revealed that, within the region predicted to encode the human ChAT protein, the only differences between the two transcripts is the presence or absence of a single exon. Omitting this exon from the 6000 nt mRNA causes a shift in the translation reading frame which would result in the synthesis of a truncated protein with a unique 9 residue carboxyl terminus. These results suggest that the regulation of human ChAT gene transcription and, perhaps, enzyme activity may be complex.

## SESSION 5: SYNTHESIS AND STORAGE OF ACH

### POSTER ABSTRACTS

P29

CHOLINERGIC CHARACTERISTICS OF AN EWING'S SARCOMA CELL LINE. Seana O'Regan and Sheela Vyas, Dépt. Neurochimie, NBCM, CNRS, 91180 Gif-sur-Yvette and INSERM U289, Hôpital de la Salpêtrière, 75651 Paris, France.

A neuroepithelial cell line derived from an Ewing sarcoma, Cohn, was examined for a neurotransmitter phenotype. Cohn cells were observed to possess NVP sensitive choline acetyltransferase (ChAT) activity (0.12 nmol/min/mg protein), but no tyrosine hydroxylase activity. Moreover, a Northern probe analysis of poly (A)<sup>+</sup>mRNA extracted from the cells revealed two ChAT mRNA species, identical to those of human brain tissue. Cohn cells also took up choline from the extracellular medium by a saturable mechanism with a  $K_m$  of 11  $\mu$ M that was reduced by half when lithium was substituted for sodium in the medium. Hemicholinium-3 acts as a competitive inhibitor of choline uptake with a  $K_i$  of 1  $\mu$ M. Following 5 min of incubation with <sup>3</sup>H-choline, only 7% of the total accumulated radioactivity was found as free <sup>3</sup>H-choline and an equivalent amount of <sup>3</sup>H-ACh was measured in the cell extract. Longer incubations did not lead to an increase in the levels of choline and ACh, although other choline metabolites involved in phospholipid and protein synthesis went up over the same time period. The endogenous ACh content of Cohn cells was 2 nmol/mg. Cohn cells thus share cholinergic characteristics with neural crest ANS progenitors.

P30

HYDROPHILIC AND AMPHIPHILIC FORMS OF CHOLINE ACETYLTRANSFERASE EXIST IN *DROSOPHILA* AND ARE ENCODED BY A SINGLE mRNA.

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The enzyme choline-O-acetyltransferase (ChAT) was found to exist in a soluble, hydrophilic, form and in a membrane-bound, amphiphilic, form in isolated nerve terminals from rat, mouse, human and *Torpedo* neuronal tissue. We showed that in the nervous system of the fly *Drosophila melanogaster* these two forms of ChAT are also present. This demonstration was based on the operational demonstration of interaction with non denaturing detergents. Sequential extraction of *Drosophila* heads produced low salt soluble (87%) and detergent soluble (6%) activities. Sedimentation analysis of detergent soluble ChAT was found to be influenced by the type of non ionic detergent used (Triton X-100 and Brij 96) whereas this was not the case for the soluble ChAT. *Drosophila* heads were also subjected to Triton X-114 fractionation that can separate hydrophilic from amphiphilic proteins. Hydrophilic and amphiphilic proteins partition in an aqueous and in a detergent phase, respectively. Using this method we found that 76% of the total ChAT activity is hydrophilic activity and 7% is amphiphilic activity. Aiming to understand how the amphiphilic form is attached to membrane, we cloned a full length ChAT cDNA from *Drosophila* heads. This cDNA was then subcloned in an expression vector in order to express it into *Xenopus laevis* oocytes. Triton X-114 fractionation of injected oocytes revealed that about 14% of the total ChAT activity expressed is amphiphilic. In control experiments, we assayed lactate dehydrogenase activity, a soluble marker. Less than 1% of it partitioned as amphiphilic activity. Moreover, Triton X-114 fractionation of non injected oocytes in the presence of low salt soluble ChAT activity extracted from *Drosophila* heads, resulted in only 3% of the enzyme partitioning as amphiphilic activity. These observations proved that the separation of proteins into hydrophilic and amphiphilic was satisfactory and that the production of significant levels of amphiphilic ChAT expressed in injected oocytes was not simply due to the contamination of the Triton X-114 phase with soluble ChAT. In conclusion, we have shown that an amphiphilic form of ChAT exists also in *Drosophila* and that hydrophilic and amphiphilic ChAT activities appeared to be encoded by a single cDNA species.

## SESSION 5: SYNTHESIS AND STORAGE OF ACh

### POSTER ABSTRACTS

- P31 CHOLINE-O-ACETYLTRANSFERASE IN RAT HIPPOCAMPAL TISSUE IS ASSOCIATED WITH MEMBRANES BY A GLYCOSYL-PHOSPHATIDYLINOSITOL ANCHOR. L.K. Smith and P.T. Carroll. Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Although most of the choline-O-acetyltransferase (ChAT) in central cholinergic nerve terminals is soluble (Fonnum, 1968), some also appears to be non-ionically associated with membranes (Benishin and Carroll, 1983). We tested the hypothesis that some of this membrane-bound, detergent soluble ChAT (D-ChAT) in rat hippocampal tissue might be linked to membranes by a glycosyl-phosphatidylinositol (GPI) anchor. ChAT appeared to possess three characteristics common to many GPI-anchored proteins: 1.) Phosphatidylinositol specific-phospholipase C (PI-PLC) selectively released it from membranes; 2.) PI-PLC treatment converted it from a detergent into a water soluble form; 3.) An antibody to an epitope on the GPI anchor (anti-CRD) reacted with the water soluble, cytosolic fraction of ChAT. We also tested the possibility that D-ChAT might be GPI-anchored intracellularly by internalizing PI-PLC into synaptosomes by DMSO freeze/thawing, and determining if it would release D-ChAT into the cytosol. Internalized PI-PLC increased the amount of ChAT in the cytosol by releasing D-ChAT from membranes. To ascertain whether an endogenous GPI-PLC might act to remove D-ChAT from membranes, we incubated a plasma membrane enriched subcellular fraction in the absence or presence of zinc, an inhibitor of GPI-PLC. Zinc abolished the temperature-dependent conversion of ChAT from a detergent into a water soluble form. Our results suggest that some of the D-ChAT is GPI-anchored intracellularly in rat hippocampal nerve terminals; also, that an endogenous GPI-PLC-like enzyme releases it from membranes into the cytosol. (Supported by NINCDS 2 RO1 NS 21289-08).

- P32 BASAL ACh SYNTHESIS IS NOT REGULATED BY MEMBRANE-BOUND CHOLINE ACETYLTRANSFERASE IN RAT HIPPOCAMPAL SYNAPTOSOMES. B.M. Schmidt & R.J. Rylett. Dept. Physiology, University of Western Ontario, London, Canada. N6A 5C1

Choline acetyltransferase catalyses the formation of ACh within cholinergic nerve terminals and appears to exist in both cytosolic (cChAT) and membrane-associated (mChAT) subcellular pools. Alteration in synaptosomal Cl<sup>-</sup> homeostasis was used as a tool to elucidate mChAT's role in regulating basal ACh synthesis. Reduction of extracellular Cl<sup>-</sup> concentration from 131 mM to 48 mM by isosmotic replacement with isethionate ions produced a selective decrease, to approximately 50% of control, of nerve terminal mChAT activity. Under these experimental conditions, there was no change in cChAT or high-affinity choline uptake activities, or in ACh synthesis. In anion substitution studies, replacement of medium Cl<sup>-</sup> (8 mM remaining) with Br<sup>-</sup> supported maintenance of synaptosomal mChAT activity better than did I<sup>-</sup> or isethionate ions. Under these conditions, high-affinity choline uptake activity and ACh synthesis were affected similarly. Incubation of synaptosomes with low concentrations of the Cl<sup>-</sup> channel blockers SITS (50  $\mu$ M) and niflumic acid (100  $\mu$ M) selectively decreased mChAT activity ( $31 \pm 16$  and  $58 \pm 14\%$  of control, respectively), with no effect on cChAT or high-affinity choline uptake activities. ACh synthesis was unchanged, even though mChAT activity was decreased in some samples (250  $\mu$ M SITS) to about 10% of control. Incubation of synaptosomes in the presence of 50  $\mu$ M muscimol, a GABA<sub>A</sub> agonist which elevates cytosolic Cl<sup>-</sup> concentration through receptor-mediated Cl<sup>-</sup> influx, increased mChAT activity to  $140 \pm 16\%$  of control, without altering cChAT or high-affinity choline uptake activities, or ACh synthesis. Thus, manipulations designed to alter neuronal Cl<sup>-</sup> homeostasis resulted in dissociation of cholinergic neurochemical parameters and allowed the first direct examination of mChAT's role in regulation of ACh synthesis. Synaptosomal mChAT activity was selectively increased to about 140% of control or decreased to about 10% of control without altering cChAT or high-affinity choline uptake activity. As ACh synthesis did not differ from control, it appears that mChAT does not play a regulatory role in biosynthesis of the neurotransmitter under basal conditions. (supported by Medical Research Council of Canada).

**SESSION 5: SYNTHESIS AND STORAGE OF ACH**

**POSTER ABSTRACTS**

- P33 **BROMOACETYLCHOLINE AND R-BROMOACETYLCARNITINE, SELECTIVE INHIBITORS OF CHOLINE AND CARNITINE-ACETYLTRANSFERASES, AND THE EVALUATION OF RAT RETINAL ACETYLTRANSFERASES.** V.E. Janson and B.V. Rama Sastry. Departments of Anesthesiology and Pharmacology, Vanderbilt Medical Center, Nashville, TN 37232-2125

Choline acetyltransferase (ChA) catalyzes the synthesis of acetylcholine (ACh) from choline and acetylcoenzyme A (ACoA) in both nervous and non-nervous tissues. Carnitine acetyltransferase (CaA) occurs in several tissues and transfers acetyl groups from ACoA to carnitine for form acetylcarnitine (ACa) and exhibits weak ChA activity. Several haloacetylcholines and haloacetylcarnitines were synthesized to develop selective inhibitors of ChA and CaA. ACh is a transmitter for some presynaptic neurons and/or amacrine cells in retina. Selective inhibitors of ChA and CaA were used in the evaluation of ChA and CaA activities in the rat retina. ChA and CaA activities were assayed by the transfer of  $^{14}\text{C}$ -acetyl group  $^{14}\text{C}$ -ACoA to choline or carnitine and estimating  $^{14}\text{C}$ -ACh or  $^{14}\text{C}$ -ACa. This study gave the following results: (a) Bromoacetylcholine (BrACh) was a selective inhibitor of purified ChA ( $\text{IC}_{50}$ ,  $5 \times 10^{-4} \text{ M}$ ); (b) R-bromoacetylcarnitine (R-BrACa) was more potent for inhibiting CaA ( $\text{IC}_{50}$ ,  $2 \times 10^{-7} \text{ M}$ ) than ChA ( $\text{IC}_{50}$ ,  $3.5 \times 10^{-5} \text{ M}$ ); (c) Rat retinal sonicate gave ChA activity of  $98 \pm 6 \text{ nmol of ACh formed/mg/10 min}$ . When the CaA was completely inhibited by R-BrACa, the activity for ChA decreased to  $47 \pm 1 \text{ nmol}$ , and this decrease was possibly due to formation of some  $^{14}\text{C}$ -ACh by CaA; (d) Rat retinal sonicate contained CaA activity of  $102 \pm 7 \text{ nmol ACa formed/mg protein/10 min}$ . This was not altered by inhibition of ChA with BrACh. This means that ChA did not use carnitine as a substrate. ChA and CaA activities did not change by dialysis of retinal sonicates at  $4^\circ\text{C}$  for 24 hrs. These observations suggest that BrACh and R-BrACa are useful for assessing correct values for ChA and CaA activities in tissues. (Supported by The Council for Tobacco Research, US HHS NIDA DA 06207, and The Study Center for Anesthesia Toxicology.)

- P34 **EFFECTS OF INTRAVENTRICULAR INJECTION OF CHOLINE ACETYLTRANSFERASE INHIBITOR, (2-BENZOYLETHYL)TRIMETHYLAMMONIUM (BETA) IN THE RAT.** B.V.R. Sastry, G. Singh, J.L. Horn and V.E. Janson. Department of Pharmacology and Anesthesiology, Vanderbilt University Medical Center, Nashville, TN 37232-2125.

A deficiency of choline acetyltransferase (ChA) and degeneration of presynaptic cholinergic neurons has been found in senile dementia of Alzheimer's type which correlates well with severity of dementia. The Committee on Animal Models for Research recommended Fischer 344 rats as a suitable model for studies of basic underlying principles governing aging and age-associated diseases. In these rats, cerebral ChA activity decreased by 16-37% in 21-27 month old rats when compared to 3 month old rats. Several ChA inhibitors have been developed for studying the effects of ChA-deficits in the rat. BETA is a strong inhibitor of ChA in vitro ( $\text{IC}_{50}$ ,  $3 \mu\text{M}$ ). In the present study rats were anesthetized with halothane delivered in a warmed humidified air/oxygen mixture. They were given an injection of BETA ( $2\text{IC}_{50}$ ,  $6 \mu\text{M}$ ) in  $50 \mu\text{l}$  of phosphate buffer ( $50 \text{ mM}$ ,  $\text{pH } 7.4$ , 30% propylene glycol) into the third ventricle using a stereotaxic frame. Rats were allowed to recover for 10 min prior to decapitation. The control group was injected with phosphate buffer containing propylene glycol. The rat brains were isolated and dissected into cerebrum, midbrain, cerebellum and medulla. The activity of the enzyme ChA was determined by a radiometric method. Clinical symptoms were observed during and after the instillation of BETA into the brain. Clinical observations following the injection of BETA showed a brief arousal response in anesthetized rats at 20-45 seconds after the injection and an increase in the rate of respiration followed by persistent depression. The ChA activity was significantly inhibited in midbrain and medulla (27% and 20% of control) respectively. These results suggest that the level of ACh at discrete sites can be altered by the use of ChA inhibitors to develop an animal model for studying the acute and chronic behavioral changes associated with neurological disorders due to brain ACh deficits. (Supported by The Study Center for Anesthesia Toxicology, The Council for Tobacco Research. USA. Inc. and US HHS NIDA DA 06207.)

SESSION 5: SYNTHESIS AND STORAGE OF ACh

POSTER ABSTRACTS

P35 STIMULATORY EFFECTS OF cAMP AND RETINOIC ACID ON ACETYLCHOLINE SYNTHESIS IN A MURINE SEPTAL CELL LINE ARE ADDITIVE.

Jan Krzysztof Blusztajn<sup>1</sup>, Ulrike Schüller<sup>1</sup>, Amy Venturini<sup>1</sup>, Darrell A. Jackson<sup>1</sup>, Henry J. Lee<sup>2</sup> and Bruce H. Wainer<sup>2</sup>.

<sup>1</sup>Boston University School of Medicine, Boston, MA 02118, & <sup>2</sup>University of Chicago, Chicago, IL 60637.

In order to study differentiated properties of brain cholinergic neurons using long term culture we have developed a cell line (SN56.B5.G4) derived from fusion of mouse postnatal day 21 septal neurons with murine neuroblastoma cells, N18TG2 [*Dev. Brain Res.* (1990) 52:219]. These cells exhibit several features of cholinergic neurons including choline acetyltransferase (CAT) activity, sodium-dependent-high-affinity uptake of choline, and depolarization-evoked ACh release [*J. Neurosci.* (1992) 12:793]. In cells treated with 1 mM of a cAMP analog, N<sup>6</sup>,O<sup>2</sup>-dibutyryl-adenosine-3'-5'-cyclic monophosphate (dbcAMP) or with 10  $\mu$ M forskolin [an activator of adenylyl cyclase] for two days, the activity of CAT and ACh content were 2-3-fold higher relative to controls. CAT activity and ACh content were also elevated up to four-fold in cells treated with 1  $\mu$ M of all trans retinoic acid (RA) for two days. These effects were time- and dose-dependent. The EC<sub>50</sub> values for dbcAMP, forskolin and RA were 1.3 mM, 0.7  $\mu$ M, and 10 nM, respectively. The effects of RA and forskolin were additive and resulted in a five-fold increase in ACh content in cells treated with 1  $\mu$ M RA and 10  $\mu$ M forskolin for two days, relative to controls. The enhancement of ACh synthesis by agents which increase intracellular cAMP levels and by RA in SN56.B5.G4 cells suggests that ACh synthesis *in vivo* may be regulated by 1) activation of receptors for neurotransmitters, hormones, or growth factors which activate adenylyl cyclase, and 2) activation of the retinoid receptors. The data also indicate that RA and elevated intracellular cAMP levels stimulate ACh production by two different mechanisms.

Supported by: NSF BNS8808942, MH46095, NS25787 & Center for Alternatives to Animal Testing grants.

## SESSION 6: ACH TURNOVER AND RELEASE

### S21. SYMPOSIUM ABSTRACTS

**ACETYLCHOLINE TURNOVER AND RELEASE. CHAIRMAN'S INTRODUCTION.** Oscar U. Scremin. West Los Angeles VA Medical Center and Department of Physiology, UCLA School of Medicine, Los Angeles CA 90024, USA.

The steady state level of acetylcholine (ACh) in brain tissue results from a dynamic equilibrium between synthesis and release of this transmitter. Synthesis of ACh in turn appears to be determined by the availability of its precursors, acetyl-CoA and choline (Ch) and the concentration of the products. Acetyl-CoA for ACh synthesis originates from pyruvate and thus requires oxidative metabolism. Ch on the other hand is supplied by net production from phospholipids, uptake of Ch derived from enzymatic hydrolysis of released ACh and plasma Ch. It has been known for long that the arteriovenous difference of Ch across brain ( $Ch_{A-V}$ ) is negative, implying a continuous loss of this base. This phenomenon has been difficult to interpret since the brain cannot synthesize Ch *de novo* at a rate sufficient to satisfy its own demand of this molecule. Recent experimentation to be reviewed in this Symposium has revealed that  $Ch_{A-V}$  can be reversed by enhancing arterial Ch concentration, resulting in Ch gain by brain. This may occur spontaneously or following brief periods of hypocapnic hypoxia, a condition that induces release of large amounts of Ch from lungs and splanchnic organs. The brain Ch loss on the other hand, can be exaggerated by brief periods of apnea or global ischemia. Since these conditions are accompanied by drastic changes in cerebral blood flow (CBF), the net exchange rate of Ch between blood and brain can only be calculated by measuring both CBF and  $Ch_{A-V}$  over prolonged periods of time. It is becoming increasingly evident that in order to understand the dynamics of ACh synthesis in the central nervous system it is necessary to evaluate carefully the balance of Ch across brain capillaries, which is in turn dependent on the interplay between release and uptake of this molecule by extracerebral tissues. These facts emphasize that cerebral cholinergic function and dysfunction cannot be considered in isolation and must be integrated with the global behavior of organ systems.

### S22 CHOLINE, A PRECURSOR OF ACETYLCHOLINE AND PHOSPHOLIPIDS IN THE BRAIN K. Löffelholz, J. Klein, A. Köppen Dept. of Pharmacology, University of Mainz, W-6500 Mainz, Germany

Cholinergic neurotransmission depends on the availability of extracellular choline (Ch), an essential nutrient. We reported previously that exogenous Ch enhances both Ch plasma level and subsequently net uptake of Ch into the brain; nevertheless, free choline of the brain was essentially kept constant by two major homeostatic mechanisms: cellular uptake with subsequent phosphorylation and incorporation into phospholipids, and outward transport from brain to blood (Klein et al., J Neurochem 55: 1231, 1990; 58: 870, 1992). Our hypothesis of brain Ch homeostasis was confirmed by studying the effects of chronic Ch deficiency and supplementation. - The efficacy of the outward transport of brain Ch as a homeostatic mechanism was studied by the microdialysis technique in awake rats. Concentric microdialysis probes (O.D. 0.24 mm) were placed into the ventral hippocampus of male Wistar rats, and Ch efflux reflecting extracellular Ch was monitored by HPLC-ECD. Nicotinamide (10 mmol/kg s.c.) which was used as a pharmacological tool to inhibit the outward transport of brain Ch, doubled Ch efflux, whereas Ch administration (20 mg/kg i.p.) caused a small and transient elevation of extracellular brain Ch in spite of a marked increase in plasma Ch. After treatment with nicotinamide, however, the same Ch injection caused a 30-fold elevation of the extracellular brain Ch (area under the curve) that lasted for 3 hours. We suggest that newly taken up Ch is rapidly eliminated from the extracellular space by cellular uptake and subsequent metabolism. Surplus Ch, free or bound, is gradually removed from the brain by outward transport. Inhibition of this outward transport (by nicotinamide) plus Ch administration are the basis for our current studies on the relationship between free Ch and ACh synthesis.

## SESSION 6: ACH TURNOVER AND RELEASE

### SYMPOSIUM ABSTRACTS

- S23 D1 AND D2 DOPAMINE RECEPTORS AND THE REGULATION OF STRIATAL IN VIVO ACETYLCHOLINE RELEASE S. CONSOLO, P. GIROTTI, M. ZAMBELLI, G. RUSSI, M. BENZI AND R. BERTOCELLI, Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy

D1 and D2 receptors subserve opposing functions on the extracellular acetylcholine (ACh) content of rat striata. Stimulation of D2 receptors inhibited ACh release in vivo, whereas stimulation of D1 receptors facilitated it. We also found that 1) the D1 receptors mediating the facilitatory effect of dopamine (DA) on ACh release are in the striatum. Thus, the D1 antagonist SCH 23390 (20  $\mu$ M) infused through the dialysis probe, or locally injected [0.45 nmol/striatum) reduced striatal ACh release to the same extent as when the drug was systemically administered; 2) changes in endogenous DA affect the D1 rather than D2 receptor mechanism. Thus, raising extracellular DA content with d-amphetamine or pargyline resulted in a time-dependent increase in striatal ACh output. The effect was fully prevented by SCH 23390 (10  $\mu$ M) infused, or administered s.c. (0.025 mg/kg). Lowering extracellular DA by inhibiting DA synthesis with  $\alpha$ -methyl-p-tyrosine ( $\alpha$ -MpT) or impairing DA vesicular uptake with reserpine resulted 16h later in a 50% decrease of basal ACh release.  $\alpha$ -MpT treatment prevented the effects of the D1 antagonist SCH 23390 but not of the D2 antagonist REM on ACh release at that time, suggesting that DA tone is depressed at the D1 receptors but not at the D2 receptors. The new model of DA-ACh interaction resulting from these data casts fresh light on the relationship between changes in DA transmission and extrapyramidal motor function.

- S24 COMMENTS ON PROBLEMS IN CHOLINE AND ACETYLCHOLINE TURNOVER AND RELEASE S. Tuček, V. Doležal and J. Říčný  
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Important progress has been achieved during recent years in the understanding of the turnover of choline and of the balance of its supply to and release from the brain (based on measurements of arterio-venous differences under varying experimental conditions), of functional variations in the release of acetylcholine (ACh) in specified areas within the CNS (based on the exploitation of the brain microdialysis technique), and of presynaptic receptors involved in the control of ACh release from the nerve terminals. Most important news will be presented by K. Löffelholz and S. Consolo in the session on Acetylcholine Turnover and Release. Related evidence obtained in our Laboratory points to the following: (a) The provision of ACh precursors is indeed an important factor in ACh homeostasis in the brain. (b) The inhibition of choline efflux from cholinergic neurons may increase their ACh stores. (c) The spontaneous non-quantal release of ACh, thought to be responsible for a large proportion of ACh turnover (at least in motor nerves), seems to be much smaller than generally believed.



SESSION 6: ACH TURNOVER AND RELEASE

POSTER ABSTRACTS

P36 THE COMBINED ADMINISTRATION OF CHOLINE AND LITHIUM ENHANCES ACETYLCHOLINE RELEASE IN THE RAT STRIATUM.

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Acetylcholine release in the striatum of the freely moving rat was measured with a transverse-type dialysis probe perfused with artificial cerebrospinal fluid containing neostigmine. Ch and ACh were measured in 0.5-min fractions by HPLC, a new sandwich-type enzyme reactor and electrochemical detection. Addition of choline chloride (30  $\mu$ M) to the perfusion medium did not affect ACh release. When lithium chloride (4 mM) was present in the perfusion fluid the effect on ACh release was variable, ranging from no effect at all to increases of about 100 %. The addition of both Ch and Li to the perfusion medium always led to increases in ACh release of at least 100 %. Our results confirm the general finding that choline by itself is not able to enhance levels, synthesis or release of ACh. In most but not all of our experiments striatal release of ACh could be enhanced by local administration of lithium chloride. We suggest that this variability is dependent on extracellular choline levels, which might fluctuate around a critical level. Indeed addition of extra Ch to the Li-containing perfusion fluid always led to increases in ACh release of even higher magnitude. The mechanism by which these increases are produced is not clear. Possibly concentrations of choline in the cholinergic nerve endings can be elevated by inhibition by lithium of trans-membrane transport of choline, in the same way as is known to occur in the red blood cell.

P37 A STUDY OF IN VIVO RELEASE AND BIOSYNTHESIS OF ACETYLCHOLINE IN RAT STRIATUM FOLLOWING CHOLINE ADMINISTRATION USING BRAIN MICRODIALYSIS.

T. Ohue, K. Koshimura, Y. Takagi, T. Shiraki and S. Miwa

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The purpose of the present study is to clarify the effects of administration of choline on in vivo release and biosynthesis of acetylcholine (ACh) in the brain. For this purpose, the change in the extracellular concentration of choline and ACh in the rat striatum were determined following intracerebroventricular administration of choline using brain microdialysis. We also determined changes in the tissue content of choline and ACh. When the striatum was dialyzed with Ringer solution containing 10  $\mu$ M physostigmine, ACh levels in dialysates rapidly and dose-dependently increased following various doses of choline, and reached maximum within 20 min. In contrast, choline levels in dialysates increased after a lag period of 20 min following the administration. When the striatum was dialyzed with physostigmine-free Ringer solution, ACh could not be detected in dialysates both before and even after choline administration. After addition of hemicholinium-3 to the perfusion fluid, the choline-induced increase in ACh levels in dialysates was abolished. Following administration of choline, the tissue content of choline and ACh increased within 20 min. These results suggest that administered choline is rapidly taken up into the intracellular compartment of the cholinergic neurons, where it enhances both release and biosynthesis of ACh.

SESSION 6: ACH TURNOVER AND RELEASE

POSTER ABSTRACTS

- P38 SEROTONIN-MEDIATED ENHANCEMENT OF *IN VIVO* ACETYLCHOLINE RELEASE BY 6R-TETRAHYDROBIOPTERIN IN THE RAT HIPPOCAMPUS USING BRAIN MICRODIALYSIS.  
T. Ohue, K. Koshimura, Y. Takagi, T. Shiraki, S. Miwa and \*Y. Watanabe.  
Department of Pharmacology, Kyoto University Faculty of Medicine, Kyoto 606, and \*Department of Neuroscience, Osaka Bioscience Institute, Suita 565, Japan.

We showed previously that 6R-tetrahydrobiopterin (6R-BH<sub>4</sub>), a common cofactor for hydroxylases of tyrosine, tryptophan and phenylalanine, has dopamine releasing action which is not dependent on dopamine biosynthesis and that intracerebroventricular injection of 6R-BH<sub>4</sub> increases acetylcholine (ACh) release in the hippocampus. Recently, we have developed a method for separation of ACh from 6R-BH<sub>4</sub>, which allows us to apply 6R-BH<sub>4</sub> to the perfusion fluid. Infusion of 6R-BH<sub>4</sub> enhanced ACh release in the hippocampus, suggesting that the action site of 6R-BH<sub>4</sub> is located in the hippocampus. In contrast, infusion of 6S-BH<sub>4</sub>, a diastereoisomer of 6R-BH<sub>4</sub>, or biopterin, an oxidized form of 6R-BH<sub>4</sub>, had no effect on ACh release. The 6R-BH<sub>4</sub>-induced increase was abolished after depletion of serotonin in the hippocampus with p-chlorophenylalanine, but not after depletion of catecholamines with  $\alpha$ -methyl-p-tyrosine. Finally, ACh release was elevated by infusion of serotonin or intraperitoneal injection of 5-hydroxytryptophan, a precursor of serotonin, which is comparable to that of 6R-BH<sub>4</sub>. These results show that 6R-BH<sub>4</sub> stimulates exocytotic ACh release in the hippocampus through action on a local serotonergic system in the hippocampus.

- P39 CHOLINE ENHANCES SCOPOLAMINE-INDUCED RELEASE OF ACETYLCHOLINE IN THE DORSAL HIPPOCAMPUS OF CONSCIOUS FREELY MOVING RATS.

Darrell A. Jackson,<sup>1,2</sup> Udo Kischka<sup>2</sup> and Richard J. Wurtman.<sup>2</sup>

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<sup>2</sup>Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.

We examined the effects of exogenous choline (30, 60, or 120 mg/kg, ip) on basal and scopolamine-evoked acetylcholine (ACh) release in awake animals using *in vivo* microdialysis. After collection of 3-4 baseline samples (15 min. each), rats received saline or choline chloride, and 4 additional samples were collected. All animals then received scopolamine hydrochloride (0.5 mg/kg, ip), and six additional samples were collected. Basal ACh release in animals receiving choline did not differ from those given saline, or from those obtained in individual rats prior to choline administration. Scopolamine alone increased average dialysate ACh levels from  $1.22 \pm 0.54$  to  $11.18 \pm 3.07$  pmol/15 min. (mean  $\pm$  SD;  $p=0.001$ ); administration of 60 mg/kg or 120 mg/kg choline doses significantly enhanced the scopolamine responses ( $p=0.002$ ,  $p=0.009$ , respectively). These results suggest that supplemental choline enhances the evoked, but not the basal release of ACh in the hippocampus of freely moving rats.

## SESSION 6: ACH TURNOVER AND RELEASE

### POSTER ABSTRACTS

- P40 **A METHODOLOGY FOR THE MEASUREMENT OF BRAIN CHOLINE BALANCE. EFFECTS OF ISCHEMIA AND APNEA.** Oscar U. Scremin and Donald J. Jenden. West Los Angeles VA Medical Center and Departments of Physiology and Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024, USA.

The cholinergic system of the brain may be particularly sensitive to ischemic or hypoxic damage. The most common sequela of cardiopulmonary resuscitation is a cognitive deficit that resembles that seen after scopolamine administration and that has been associated with the impairment of function of cholinergic cells. Furthermore, the cognitive deficit of multi-infarct dementia is sometimes indistinguishable from that of Alzheimer's disease, a condition in which a cholinergic dysfunction is a well established fact. It has been proposed that losses of Ch associated with these conditions may underlie a cholinergic deficit. We have developed a methodology for the study of the balance of Ch across brain ( $Ch_{BAL}$ ) that involves cannulation of the retrogleneid vein, the main venous outflow channel of the forebrain, and measurement of cerebral blood flow (CBF) and arteriovenous difference for Ch ( $Ch_{A-V}$ ) in mechanically ventilated rats. Integration of the product of CBF and  $Ch_{A-V}$  over time estimated  $Ch_{BAL}$  (nmoles). The results showed that after 60 min of recirculation following transient forebrain ischemia, induced by occlusion of vertebral and carotid arteries,  $Ch_{BAL}$  amounted to  $-48.8 \pm 9$  nmoles, a loss 7 times greater than that observed during a comparable period in non-ischemic animals ( $-7 \pm 2.3$  nmoles). Given that the territory drained by the retrogleneid vein in our preparations averages 0.6 g, the loss during the first hour of reperfusion results in an expected drop of free Ch concentration of  $81.3 \text{ nmoles} \cdot \text{g}^{-1}$ . Apnea of 90 seconds duration induced a  $Ch_{BAL}$  after 60 min of  $-12.05 \pm 3.57$  nmoles. When a second episode was repeated 60 min after the first, the negative  $Ch_{BAL}$  was considerably enhanced amounting to  $-39.3 \pm 7.2$  nmoles after the 90 min that followed the second episode. In conclusion, apnea and ischemia can mediate long lasting negative balance of Ch in brain tissue with a potential for disturbing ACh and phospholipid synthesis and availability. Supported by the US Department of Veterans Affairs and USPHS MH 17691.

- P41 **Dopamine Has Dual Actions on Acetylcholine Release in the Striatum: Stimulation and Inhibition**

T. Shiraki<sup>1</sup>, T. Ohue<sup>2</sup>, K. Koshimura<sup>2</sup>, Y. Takagi<sup>2</sup>, S. Miwa<sup>2</sup>, S. Kobayashi<sup>1</sup> and T. Masaki<sup>2</sup>

<sup>1</sup>Human and Environmental Studies, <sup>2</sup>Faculty of Medicine, Kyoto University, Kyoto 606, Japan.

To clarify the *in vivo* dopaminergic regulation of acetylcholine (ACh) release, we examined the effects of selective agonists of D<sub>1</sub> and D<sub>2</sub> dopamine (DA) receptors, in addition to those of DA itself on ACh release in the rat striatum using *in vivo* brain microdialysis. ACh recovered in dialysates was determined using a column-switching HPLC combined with electrochemical detector. Administration of a D<sub>1</sub> agonist (SKF38393; final concentration, 5-500  $\mu\text{M}$ ) to the perfusion fluid, increased ACh levels in striatal dialysates. The effects of a D<sub>2</sub> agonist (Quinpirole) on ACh levels in dialysates are biphasic: at 5  $\mu\text{M}$ , it reduced, whereas at 500  $\mu\text{M}$ , it increased them, with ACh levels being unchanged at 100  $\mu\text{M}$ . After depletion of dopamine by reserpine, D<sub>2</sub> agonist-induced enhancement of ACh release at high concentrations of D<sub>2</sub> agonist was inhibited, but other effects were observed similar to those under normal condition. These results suggest that the site of action of D<sub>1</sub> agonist was not primarily localized in dopaminergic neurons and that D<sub>2</sub> receptors were localized on at least dopaminergic terminals. Administration of DA reduced ACh levels in dialysates at lower concentrations (100 and 500  $\mu\text{M}$ ), whereas it augmented them at higher concentrations (1 and 2 mM). These results suggest that DA reduce ACh release at lower concentrations through activation of D<sub>2</sub> receptors on cholinergic neurons, and that enhancement of ACh release by DA at higher concentration is mediated by activation of D<sub>1</sub> receptors on cholinergic neurons and/or D<sub>2</sub> receptors on dopaminergic terminals.

## SESSION 6: ACH TURNOVER AND RELEASE

### POSTER ABSTRACTS

**P42 A NOVEL CLASS OF GALANIN RECEPTOR ANTAGONISTS WHICH BLOCK THE GALANIN-MEDIATED INHIBITION OF STIMULATED ACETYLCHOLINE RELEASE IN THE RAT VENTRAL HIPPOCAMPUS.**

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The 29 amino acid long neuropeptide galanin has been shown to coexist with acetylcholine in a subpopulation of neurons located in the basal forebrain of rat, monkey, and man. These neurons are known to provide a major cholinergic innervation to neocortical areas. In particular, the septal-diagonal band complex provides most of the cholinergic innervation to the hippocampal formation. High affinity binding sites for [<sup>125</sup>I]galanin have been characterized specifically in the rat ventral hippocampus, where galanin exerts a multiple control of cholinergic activity, by inhibiting the evoked release of acetylcholine and the muscarinic receptor-mediated stimulation of phosphoinositide turnover. Recent data indicate that galanin is a potential physiological modulator of cholinergic function in the hippocampus of primates, adding further consistency to the hypothesis that this neuropeptide may exert the same type of modulation in the human brain. This issue is of particular interest, since galanin has been proposed to play some role in cognitive functions as well as to be involved in neuropathological disorders affecting the cholinergic system, like senile dementia of the Alzheimer's type. Here we present data showing that synthetic chimeric peptides composed by the biologically active N-terminal portion of galanin, galanin(1-13), and C-terminal portions of other bioactive peptides act as high affinity galanin receptor antagonists, blocking the galanin-mediated inhibition of scopolamine-stimulated release of acetylcholine, measured in the ventral hippocampus of freely moving rats. By virtue of increasing cholinergic transmission in the hippocampus, galanin receptor antagonists may prove useful therapeutic agents in the treatment of Alzheimer's disease.

**P43 EXCITATORY AND INHIBITORY MODULATION OF THE SEPTO-HIPPOCAMPAL CHOLINERGIC PATHWAY: AN "IN VIVO" MICRODIALYSIS STUDY.**

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The septo-hippocampal cholinergic pathway originating in the medial septum provides the main cholinergic input to the hippocampus. In order to investigate the functional control of this pathway we studied the modulatory effect of excitatory and inhibitory aminoacidergic pathways on hippocampal ACh release. Extracellular levels of ACh were determined by means of the transversal microdialysis technique in freely moving rats. ACh in the dialysate was quantified by an HPLC method. I.c.v. administration of the NMDA antagonists CPP, AP-7 and MK801 increased ACh release from the hippocampus while the non-NMDA antagonist DNQX was ineffective in the dose-range tested (0.25-20 nmol). The effect of CPP (dose-dependent in the range 1-50 nmol; maximum effect about +500%) was blocked by TTX (0.5  $\mu$ M) and abolished by i.c.v. injection of the GABA<sub>A</sub> agonist muscimol. CPP (100  $\mu$ M) had no effect when applied in the hippocampus via the dialysis tubing, suggesting that presynaptic NMDA receptors do not affect ACh release from the septo-hippocampal pathway. However, the same dose of CPP administered in the septum increased ACh release from the hippocampus and this effect was abolished by concomitant administration of muscimol in the septum. These results suggest that the cholinergic septo-hippocampal pathway is modulated at septal level by GABAergic neurones activated by NMDA receptors.

Investigation supported by a Grant from M.U.R.S.T., Italy.

## SESSION 6: ACH TURNOVER AND RELEASE

### POSTER ABSTRACTS

#### P44 CYTOKINES AND THE MODULATION OF CHOLINERGIC TRANSMISSION:

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Classical immunoregulators, i.e. cytokines, most likely play pivotal roles in the normal development and function of the nervous system. On the other hand, growing evidence suggests also an involvement in both pathologic processes leading to neurologic symptoms as well as in the generation of adverse symptoms during immunotherapy. Some cytokines may be synthesized by cells intrinsic to the CNS. Alternatively, cytokines being able to cross the blood-brain barrier may serve as shared effectors in the communication between the immune system and the CNS, either triggering and controlling effects in remote tissues or providing humoral feedbacks. One domain of demonstrated cytokine effects deals with the control of neuronal activity. We studied the effect of interleukin-2 (IL-2) on the release of endogenous acetylcholine (ACh) from superfused rat brain slices. IL-2 inhibited the K(+)-evoked ACh release from hippocampal slices, with a pronounced activity at a concentration of 1 nM. Toxic effects could be excluded for the duration of the experiments. IL-2 showed a similar reduction in ACh release in tissue slices from frontal cortex, but was ineffective on samples from the striatum and the parietal cortex. On the contrary, IL-2 at 0.1 pM induced a transient increase in the evoked hippocampal ACh release, resulting in a biphasic profile of the dose-response relation. The data support the notion that IL-2 exhibits neuromodulatory potential, at least in respect to certain populations of cholinergic neurons. The results on IL-2 (i) will be compared to ACh release studies involving a number of other cytokines, among those IL-1, IL-3, IL-5, and IL-6, and (ii) will be discussed with regard to the conditions under which (extraneous) IL-2 may become effective in the brain. (Supported by the FRSQ, the MRCC, and the HFSPQ.)

#### P45 THE POSSIBLE ROLE OF ACETYLCHOLINE IN ONTOGENESIS.

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Since the association of congenital anomalies with administration of Thalidomide in pregnancy, numerous reports of a possible causative relationship between neurotropic drugs and malformations have appeared in the literature. The inductive role of neuroectoderm during early embryonic differentiation has long been established; a number of organizer centres, directly related to the primary organiser are recognised for the centrally located optic cups, ear vesicles and nasal pits. It is possible that the precociously developed nervous system in the embryo has other inductive roles: in particular, the neural tube "programmes" the limb developing potential of the lateral mesoderm. In the New Zealand White Rabbit, it has been shown that the anticholinergics Scopolamine (approximately 100 ug/kg) reduced the choline levels in the placenta and fetus but not the acetylcholine levels. Orphenadrine (approximately 24 mg/kg) reduced acetylcholine and choline levels in the fetus and choline levels in the placenta. Doxylamine succinate (10 mg/kg) reduced the acetylcholine levels in the fetus and choline levels in the placenta. The placenta is a fetal organ and the significance of acetylcholine production by the placenta is as yet unknown. The reduction of acetylcholine levels in the fetus exposed to drugs with an anticholinergic action may be significant in the production of malformations. A Monoclonal antibody is being used to determine the receptor sites of acetylcholine in the early embryo in an endeavour to determine the role of acetylcholine in development.

## SESSION 7: MOLECULAR ASPECTS OF ACH RELEASE

### SYMPOSIUM ABSTRACTS

#### S25 MOLECULAR ASPECTS OF ACh RELEASE

Georgina Rodríguez de Lores Arnaiz

Instituto de Biología Celular, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, 1121-Buenos Aires, Argentina.

Modulation of ACh release is of physiological and also pharmacological importance. Subcellular fractionation studies have shown that brain acetylcholine (ACh) is firmly bound to the synaptic vesicles, involved in neurotransmitter quantal release. However, the existence of an extravesicular ACh pool, related to a cytoplasmic pool has also been suggested. ACh is released by electrical stimulation, by high  $K^+$  depolarization or by interference with sodium channels. According to the calcium hypothesis, the increase of  $Ca^{2+}$  in the axoplasm is crucial for neurotransmitter release. Since ACh release may occur in the absence of  $Ca^{2+}$ , it would be of great interest to correlate vesicular and extravesicular ACh pools with  $Ca^{2+}$  dependent and  $Ca^{2+}$ -independent release, as well as its possible physiological significance. In several experimental models a correlation between  $Na^+, K^+$ -ATPase inhibition and ACh release has been shown, which probably is non-quantal and involves the nonvesicular ACh pool. Even though choline is uptaken by nerve endings, ACh itself is not, which indicates that ACh action is not arrested by the well-recognized uptake mechanism demonstrated for several other neurotransmitters. Last but not least, auto- and heteroreceptors as well as phosphorylation-dephosphorylation of specific proteins may play a major role in the regulation of ACh release.

#### S26 MEDIATOPHORE PERFORMS THE TRANSLOCATION STEP OF THE RELEASE MECHANISM.

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Département de Neurochimie. CNRS. 91198 Gif sur Yvette Cedex. France.

Mediatophore is a nerve terminal membrane protein that translocates transmitter upon calcium action. It is an oligomer of a 15 Kd proteolipid reaching a molecular weight of about 220 Kd. The 15 Kd subunit was completely sequenced using molecular biology methods. The messenger RNA of the mediatophore was translated in *Xenopus* oocytes and the 15 Kd protein produced endowed the oocyte membrane with a calcium dependent ACh release mechanism. Most of the essential features of the natural release mechanism: calcium activation, desensitization and selectivity, were also found on reconstituted mediatophore. In addition, pharmacological actions and structural modifications appear to be common to the release mechanism and to the mediatophore. It is therefore established that mediatophore is directly involved in the ACh translocation from nerve terminals.

## SESSION 7: MOLECULAR ASPECTS OF ACH RELEASE

### SYMPOSIUM ABSTRACTS

S27

#### $\alpha$ -LATROTOXIN RECEPTOR FUNCTION IN NEUROTRANSMITTER RELEASE

A. G. Petrenko, M. Geppert, Y. A. Ushkavyov, Y. Hata and T. C. Südhof

UT Southwestern Medical Center at Dallas

$\alpha$ -Latrotoxin is a neurotoxin isolated from black widow spider venom that causes massive neurotransmitter release from vertebrate synaptic nerve endings. Neurotransmitters are packaged in synaptic vesicles and released by synaptic vesicle exocytosis.  $\alpha$ -Latrotoxin binding to nerve endings cause  $\text{Ca}^{2+}$  independent fusion of synaptic vesicles with the plasma membrane. Using sequences obtained from purified  $\alpha$ -Latrotoxin receptor, we have cloned a new family of cell surface receptors that may have a function in nerve terminal organization. The implications of the structures and properties of these receptors for synapse function will be discussed.

#### S28 UNSETTLED QUESTIONS ABOUT ACETYLCHOLINE RELEASE

Y. Dunant, A. Cavalli, Ph. Corrèges, L. Eder-Colli, R. Girod, F. Loctin and A. Parducz

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Release is quantal at the neuromuscular and other junctions but there is evidence that the quanta are composed of subunits. Several presynaptic proteins have been characterized and cloned but their precise function during or after the passage of a nerve impulse is still unclear. Vesicle openings were seen in the presynaptic membrane during ACh release under certain conditions but not in others.

Some of these questions are being addressed by using the *Torpedo* electric organ. **Quanta** of ca. 10'000 ACh molecules are produced on stimulation. They act on adjacent fields of the postsynaptic membrane (~ 800 nm apart). Each quantum is composed of preferential number of 10 subunits.

A brief tetanic stimulation elicits a net accumulation of **calcium** in axon endings. The calcium content of synaptic vesicles does not increase during the tetanus but only 1 min later. Ultrarapid freezing showed that the passage of a single nerve impulse. ACh release was accompanied by an abrupt (2-3 ms) increase in the number of intramembrane particles in the presynaptic plasmalemma. No vesicle openings were observed during impulse transmission in this synapse. In contrast, the number of vesicle openings significantly raised after activity to reach a maximum at one min after the end of the tetanus. Thus, synaptic vesicles seem to sequester calcium after activity and extrude it by exocytosis.

Whole-cell recording with an embryonic myocyte was used to demonstrate that isolated **synaptosomes** retain their physiological properties of adhering to postsynaptic membrane and of releasing ACh in a quantal manner (with M.m. Poo, Columbia, NY). Also, the ACh release mechanism was expressed in **Xenopus oocytes** injected with mRNAs from cholinergic neurons. The primed oocytes (but not controls) released the transmitter like in natural synapses. Antisens probes raised against the mediatophore protein were injected together with the mRNAs. They caused in oocytes a parallel decrease in the rate of ACh release and in the expression of the mediatophore protein (with M. Israël, F. Meunier, N. Morel and coll.).

## POSTER ABSTRACTS

P46

DIFFERENTIAL EFFECTS OF PRESYNAPTIC PHOSPHOLIPASE A<sub>2</sub> NEUROTOXINS ON *TORPEDO* SYNAPTOSOMES

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The effects of several phospholipase A<sub>2</sub> presynaptic neurotoxins ( $\beta$ -neurotoxins) from snake venoms were examined on purely cholinergic synaptosomes from *Torpedo* electric organ.

**Crotoxin** is made of two subunits: a weakly toxic phospholipase A<sub>2</sub> (CB) and a non enzymatic chaperon (CA). Both crotoxin and CB elicited a rapid and dose-dependent acetylcholine release associated with a depolarization of the preparation. Subsequent evoked acetylcholine release was reduced. These effects were dependent on the integrity of the phospholipase A<sub>2</sub> activity of the toxin: they disappeared when activating Ca<sup>2+</sup> ions were replaced by inhibiting Ba<sup>2+</sup> ions or when the active site of CB was alkylated with *p*-bromophenacyl bromide. Isolated CA was inactive but it prevented the lytic effect of CB observed at high concentrations.  $\beta$ -**bungarotoxin**, another very potent  $\beta$ -neurotoxin, released little acetylcholine, did not affect calcium ionophore-evoked acetylcholine release but significantly reduced high-K<sup>+</sup>-induced release. The single chain  $\beta$ -neurotoxin **agkistrodotoxin** presents 80% amino acid similarity with CB. It behaved like CB, through a mostly lytic mechanism. Moreover, CA was able to complex with and to behave as a chaperon toward agkistrodotoxin.

The obvious differences of effect of the various  $\beta$ -neurotoxins suggest they exert their specific actions on the excitation-secretion coupling process by different mechanisms. Binding studies of iodinated crotoxin have been undertaken to identify the molecule(s) of the presynaptic membrane responsible for the targeting of  $\beta$ -neurotoxins on nervous structures.

- P47 **FEEDBACK REGULATION OF ACETYLCHOLINE RELEASE AT INDIVIDUAL ENDPLATES IN EXPERIMENTAL MYASTHENIA GRAVIS.** J.J. Plomp, G.Th.H. van Kempen, P.C. Molenaar, L.F.G.M. Hesselmanns<sup>1</sup> and F.G.I. Jennekens<sup>1</sup>. Division of Physiological Pharmacology, Department of Physiology, Sylvius Laboratory, Leiden, and <sup>1</sup>Laboratory of Neuromuscular Diseases, University Hospital, Utrecht, The Netherlands.

Rats were chronically treated with  $\alpha$ -bungarotoxin ( $\alpha$ BTX), which caused weakness, especially of the facial muscles. Afterwards the effect of this procedure on the ACh receptors and the transmitter release was studied in their diaphragms *in vitro*. In toxin-treated animals the binding of [<sup>125</sup>I] $\alpha$ BTX to endplates was reduced to 58%. Choline acetyltransferase activity and the size of endplates, as indicated by staining for acetylcholinesterase and synaptophysin, were unchanged. The ACh release (measured with HPLC) evoked by 3 Hz stimulation was increased to 175%. The amplitude of endplate potentials (EPPs), recorded in the presence of  $\mu$ -conotoxin in order to suppress muscle spikes, was reduced, although to a lesser extent than that of the miniature endplate potentials (MEPPs). The mean quantal contents of the EPPs was increased to 154%. Within individual muscles from animals of  $\alpha$ BTX-treated rats there was an inverse relationship between the quantal content of an endplate and its MEPP amplitude. The quantal content in some endplates was increased up to 250% compared to the mean of the controls. Three hours after a single injection of  $\alpha$ BTX the amplitude of the MEPPs was reduced to about 60% but no increase of the quantal content was found. During the first few days of  $\alpha$ BTX-treatment the quantal content gradually increased until a plateau was reached after 20 - 30 days. The results suggest the existence of an adaptive mechanism, operating at individual endplates of normal size, in which retrograde signals at the motor nerve terminals modulate ACh release when neuromuscular transmission is endangered by block of ACh receptors.



## SESSION 7: MOLECULAR ASPECTS OF ACH RELEASE

### POSTER ABSTRACTS

- P48 PROTEIN PHOSPHORYLATION AND THE INHIBITION OF ACETYLCHOLINE RELEASE.  
Karen E. Asermely, Gerald H. Sterling and John J. O'Neill.  
Department of Pharmacology, Temple Univ School of Medicine, Philadelphia, PA  
There is considerable evidence supporting the role of protein phosphorylation/dephosphorylation in the regulation of neuronal function (Greengard, 1978). Phosphorylation of neuron specific proteins, e.g., Synapsin and Synaptophysin, is believed to be involved in synaptic transmission. In this study concerning factors regulating presynaptic cholinergic function, the effect on protein phosphorylation of Botulinum neurotoxin A (BoNT), a potent inhibitor of acetylcholine release, was examined. Synaptosomes from rat cerebral cortex were preincubated for 20 min with BoNT followed by a 45 min period with P<sub>32</sub>-orthophosphate. Proteins were separated by SDS-PAGE Gels with radioactivity identified by autoradiography and counted. BoNT increased the steady state phosphorylation of several neuron specific proteins, e.g., the vesicle specific 38 kDa protein, Synaptophysin which was confirmed by Western blot; a 43 kDa protein (possibly B50), associated with neurotransmitter release; and an unidentified 29 kDa component. The presence of BoNT also increased the incorporation of P<sub>32</sub> into a doublet of 80-86 kDa, resembling the Synapsins, which depend on cAMP dependent kinase for phosphorylation (Ueda & J Greengard, 1977). 4-Aminopyridine (0.1 mM), a potent epileptogenic agent, known to reverse BoNT induced inhibition of ACh release, restores steady state phosphorylation toward control levels. Since the paralytic effects of BoNT are persistent, the initial changes in protein phosphorylation states may provide important insights into presynaptic factors regulating cholinergic neurotransmission.
- P49 CHARACTERIZATION OF AN ANTIGEN POSSIBLY INVOLVED IN THE RELEASE OF ACETYLCHOLINE IN TORPEDO.  
L. Eder-Colli, J. Medilanski, N. Pellegrinelli, A. Cavalli, Y. Dunant and F. Locsin.  
Department of Pharmacology, C.M.U., 1211 GENEVA 4, Switzerland.  
A monoclonal antibody (McAB 8/38A) raised against the plasma membrane of nerve terminals isolated from the *Torpedo* electric organ, was found to inhibit acetylcholine (ACh) release induced in synaptosomes. In indirect immunofluorescence studies on the *Torpedo* electric organ and brain electric lobe (which contains cell bodies of cholinergic nerves innervating the electric organ), the McAB stained specifically the ventral side of the layered electrocytes and the cell bodies, respectively. On immunoblots of synaptosomes, the McAB labeled bands at 135 and 66 kDa. The 66 kDa polypeptide appears to exist as a monomer and as a dimer. The antigen was much less concentrated in electric nerves and lobes than in synaptosomes. The staining intensity of the antigen was significantly decreased on immunoblots of synaptosomes prepared from electric organ the electric nerves of which had been previously ligatured (i.e., denervated electric organ). The antigen can be solubilized from synaptosomal plasma membranes using mild non-ionic detergent. Treatment of intact synaptosomes with increasing concentrations of trypsin induced a progressive degradation of the antigen into lower molecular weight components but no significant loss of antigenicity. This indicated that the antigen is exposed outside the nerve terminals. It is not solubilized by the proteolytic treatment and the epitope to which binds the McAB is not cleaved by trypsin. Poly A<sup>+</sup> RNA isolated from *Torpedo* electric lobes was injected in *Xenopus laevis* oocytes. After days incubation at 18 °C, the antigen was found to be expressed. Such injected oocytes which were reported to release ACh in a calcium dependent manner (Cavalli et al. EMBO J., 1991, 10, 1671), will be used to further analyze the way the antibody affects ACh release.

## SESSION 8: TROPHIC INTERACTIONS

### SYMPOSIUM ABSTRACTS

- S29 **Trophic Interactions: Is NGF a Neurotrophic Factor for Basal Forebrain Cholinergic Neurons?** Fred H. Gage, Department of Neuroscience, University of California, San Diego, La Jolla, CA 92093-0627

Considerable data has been generated demonstrating a strong relationship between Nerve Growth Factor (NGF) and its family members, and the cholinergic neurons of the basal forebrain. NGF is said to be a neurotrophic factor for basal forebrain cholinergic neurons. However, a strict interpretation of the trophic hypotheses dictates that a subpopulation of neurons are dependent on a neurotrophic factor for proper development. This requirement has not been proven for any molecule on cholinergic neurons. A popular extension of the hypotheses is that a neurotrophic factor maintains the survival of an adult subpopulation of neurons, and that interruption of neurotrophic factor with age or disease will cause neuronal degeneration. Neither of these corollaries has been conclusively documented for cholinergic neurons. However, exogenous NGF can selectively effect intact young adult and aged cholinergic neurons. Furthermore, exogenous NGF can support the survival of young cholinergic neurons in vitro, prevent death of axotomized adult and aged cholinergic neurons, and induce regeneration of degenerating adult cholinergic neurons. Much more remains to be uncovered concerning the trophic interactions of NGF and the basal forebrain cholinergic neurons. The cellular and molecular mechanism through which NGF and its family members effect cholinergic neurons is presently under investigation. As important, however, the full range of biological effects of NGF, including whether NGF is a neurotrophic factor, remains to be determined.

- S30 **NGF AFFECTS THE CHOLINERGIC NEUROCHEMISTRY AND BEHAVIOR OF AGED RATS.** Lawrence R. Williams, CNS Diseases Research, The Upjohn Company, Kalamazoo, MI, 49001

Infusion of exogenous NGF stimulates choline acetyltransferase (ChAT) enzyme activity, high affinity choline uptake (HACU), and acetylcholine (ACh) release in the basal forebrain, cerebral cortex, hippocampus, and striatum of normal young adult Fischer 344 male rats. We have used this phenomenon to examine the regulation of cholinergic neurotransmission in young animals, and both neurochemical and behavioral dysfunction/stimulation in aged animals. There is a differential age-related regulation of ChAT, HACU, and ACh release by the cholinergic neurons in the basal forebrain and striatum. The pattern of cholinergic stimulation by NGF also indicates an age-related differential regulation of cholinergic markers between specific brain areas; the largest effects are found in the striatum. Regional changes in the levels of ACh release in aged or NGF-treated rats correlate more with the alterations in ChAT activity than of HACU. An increased electrophysiologic synaptic efficacy is observed in NGF treated animals. The stimulations in neurochemistry and electrophysiology correlate with the effects of NGF in both motor and memory behavioral tasks; both positive and negative behavioral effects are observed depending on the paradigm. Both the electrophysiologic and behavioral effects could be due simply to stimulations in ACh synthesis and release, but may also be mediated by a plasticity in synaptic structure mediated directly by NGF or through induction by stimulated ACh release. In fact, NGF treatment results in an increase in the number of dendritic spines in the cortex of aged rats.

## SESSION 8: TROPHIC INTERACTIONS

### SYMPOSIUM ABSTRACTS

- S31 **NEUROTROPHIN CONTROL OF CHOLINERGIC NEURON FUNCTION AND SURVIVAL.**  
Franz Hefti, Dalia Araujo, Klaus D. Beck, Beat Knusel, Paul A. Lapchak, and Hans R. Widmer. Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089, USA.

Differentiation of basal forebrain cholinergic neurons in culture is stimulated by nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). Based on these studies protective effects of BDNF on septo-hippocampal cholinergic neurons after injury were compared with those of NGF. Chronic intraventricular administration of BDNF to adult rats with partial transections of the fimbria significantly reduced axotomy-induced degenerative changes of the cholinergic cells in the basal forebrain. NGF and BDNF had quantitatively different effects with BDNF sustaining only part of the population of cholinergic neurons affected by the lesion, whereas the entire population was protected by NGF treatment. Partial fimbrial transections reduced hippocampal high affinity choline uptake, ChAT activity, and [<sup>3</sup>H]ACh synthesis. The lesioned induced decreases of these parameters of presynaptic cholinergic function in the hippocampus were attenuated by NGF but not BDNF treatment. Both NGF and BDNF produced significant reductions in body weight. Further analysis of the effects of NGF treatment on cholinergic function showed that neither the partial fimbrial transections nor the NGF treatment affected the densities of muscarinic receptors in the hippocampus. However, the muscarinic M1-receptor mediated response, inositol triphosphate production by hippocampal slices was increased on the lesioned side of animals treated with a control protein, and this lesion-induced supersensitivity of muscarinic receptor function was prevented by NGF treatment. In summary, the findings indicate that chronic NGF treatment-mediated increases in presynaptic function of hippocampal cholinergic neurons axons surviving partial fimbrial transections translate into functional changes at the level of postsynaptic muscarinic receptors. Comparison of BDNF and NGF effects revealed that NGF remains the agent of choice when attempting to protect cholinergic neurons from injury-induced degeneration in the adult brain.

- S32 **MODELLING CNS CHOLINERGIC NEURONS WITH TROPHIC AGENTS, A DISCUSSION**  
**A.C.CUELLO**  
Department of Pharmacology Therapeutics, McGill University, Montreal, Canada

The cellular and subcellular distribution of nerve growth factor (NGF)-receptor (r) immunoreactive sites, in forebrain cholinergic neurons, suggests a functional role for these neurotrophins in the adult fully differentiated CNS. In this session, *IN VIVO* evidence will be presented demonstrating that cholinergic neurons of the nucleus basalis magnocellularis (nbm) and of the medial septum, in both rodents and primates, are responsive to NGF. These cells undergo significant retrograde atrophy following cortical infarctions or sectioning of the fimbria-fornix. The significance and extent of trophic factor-induced attenuation of cholinergic deficits, in these experimental models, will be reviewed. There is furthermore, increasing evidence indicating that, in lesioned animals, trophic factors elicit an important remodelling of CNS cholinergic terminal networks and presynaptic sites in remaining target areas. This notion is supported by recent neurochemical studies and ultrastructural investigations combined with image analysis of choline acetyltransferase immunoreactive sites. Some of these NGF-induced responses are facilitated by the administration of sialogangliosides, both in rodents and primates.

## SESSION 8: TROPHIC INTERACTIONS

### POSTER ABSTRACTS

- P50 **Effects of Acidic fibroblast growth factor in cholinergic neurons of nucleus basalis magnocellularis and in a spatial memory task following cortical devascularization**  
B.C.Figueiredo, D Maysinger, P Piccardo, PBS Clarke and AC Cuello Dept of Pharmacology and Therapeutics, McGill University, Montreal, Canada.

The ability of acidic fibroblast growth factor (aFGF) to elicit a trophic response tested *IN VITRO* and *IN VIVO*. In rat septal cultures it was observed that the addition of aFGF (0.1 µg/ml) moderately increased the staining and length of glial processes immunostained for glial fibrillary acid protein (GFAP). *IN VIVO* studies were conducted in rats trained in a spatial memory task (Morris water maze) which were submitted to unilateral cortical devascularization. The lesion results in partial, unilateral, infarction of the neocortex and in a retrograde degeneration of the nucleus basalis magnocellularis (nBM) as shown by ChAT immunostaining. Animals were tested 24 days post-lesion for retention of the memory tasks and were sacrificed at 30 days. Intraventricular administration of aFGF (12 µg/day for 7 days via minipump), starting just before lesion reduced NBM cholinergic cell degeneration as demonstrated by the image analysis of cross sectional area of cell body and fibre length. The treatment prevented the lesion-induced impairment in the memory retention test. The most striking difference in the memory retention test between aFGF-treated (or sham-operated) and vehicle-treated decorticated subjects was observed in the trials on day one. Of these initial trials, the first showed the most significant differences. Supported by Centre of Excellence and Medical Research Council (Canada).

- P51 **ULTRASTRUCTURAL EFFECTS OF NGF AND/OR GM1 TREATMENT ON THE CORTICAL CHOLINERGIC INNERVATION IN THE ADULT LESIONED RAT BRAIN.** L. Garofalo, A. Ribeiro-da-Silva and A.C. Cuello. Dept. Pharmacology, McGill University, Montréal, Québec, Canada.

Although a number of studies have now demonstrated that NGF and/or GM1 can serve as neuroprotective agents for the adult mammalian central nervous system, information regarding their effects at the ultrastructural level is scant. Thus, we examined, using electron microscopic ChAT immunocytochemistry, the effects of NGF and/or GM1 treatment on cortical ChAT immunoreactive (ChAT-IR) presynaptic terminal morphology. In adult rats, unilateral devascularizing cortical lesions cause cholinergic deficits in the nucleus basalis magnocellularis and also diminish cholinergic fiber length, varicosity area and synaptic incidence in the remaining cortex adjacent to the lesion site. These cholinergic deficits are prevented by NGF treatment which furthermore, induces a hypertrophy of cortical ChAT-IR varicosities (PNAS 89, 2639-2643, 1992). Recently, the effects of GM1 treatment alone or when administered together with NGF was also examined (Garofalo et al., Society for Neuroscience Abstract, 1992). ChAT immunocytochemical electron microscopic analysis combined with image analysis quantification revealed that in contrast to NGF, GM1 treatment did not increase the mean cross sectional area of cortical cholinergic terminals in layer V of lesioned animals. However, it did significantly augment the NGF induced increase in ChAT-IR terminal cross sectional area. To further assess NGF and GM1 effects on cholinergic varicosities, representative boutons were examined serially and reconstructed with the aid of an image analysis system (Quantimet 920). In control unoperated rats the mean total volume of cholinergic varicosities in cortical layer V was  $0.171 \pm 0.038 \mu\text{m}^3$ . Significant decreases of 45% and 20%, respectively were noted in varicosity volume in lesioned vehicle or GM1 treated rats. By contrast NGF treatment augmented varicosity volume to 210% of control values. This presynaptic terminal growth was more pronounced (254% of control) in rats which received both NGF and GM1. We also assessed whether synaptic area was affected by these treatments. As previously shown, this lesion significantly reduces the percentage of cortical boutons which are synaptic. Lesioned rats which received GM1 treatment maintained control number of synapses while, the percent of varicosities with synaptic contacts rose to approximately 200% of control values in both NGF and NGF/GM1 treated rats. Furthermore, a trend towards an increase in synaptic area per bouton was noted in these trophic factor treated rats. This work indicates that NGF treatment can significantly augment cholinergic innervation in the adult rat lesioned brain and that this effect can be potentiated by the monosialoganglioside GM1. Supported by MRC (Canada) and the NCE Network for Neural Regeneration and Recovery (McGill Node 111). L.G. received an FRSQ studentship.

## SESSION 8: TROPHIC INTERACTIONS

### POSTER ABSTRACTS

#### P52 LONG TERM PROTECTIVE EFFECTS OF HUMAN RECOMBINANT NERVE GROWTH FACTOR AND MONOSIALOGLANGLIOSIDE GM1 TREATMENT ON MONKEY NUCLEUS BASALIS CHOLINERGIC NEURONS AFTER NEOCORTICAL INFARCTION.

P. Liberini<sup>1</sup>, D. Maysinger<sup>1</sup>, E.P. Pioro<sup>1</sup>, F.R. Ervin<sup>2</sup>, and A.C. Cuello<sup>1</sup>. Department of Pharmacology & Therapeutics<sup>1</sup> and Department of Psychiatry<sup>2</sup>, McGill University, Montreal, P.Q., Canada, H3G 1Y6.

In the present study, the biochemical and morphological damage which occurs in the rat nucleus basalis after neocortical infarction has been reproduced in nonhuman primate (*Cercopithecus aethiops*). Thirty adult male monkeys randomly divided in four groups underwent surgeries and treatment with recombinant human nerve growth factor (rhNGF), alone or in combination with the monosialoganglioside GM1 (7 sham operated, 7 lesioned/vehicle-treated, 8 lesioned/rhNGF-treated, 8 lesioned/rhNGF + GM1-treated). In lesioned animals the surgical procedures consisted in cauterization of pial blood vessels supplying the gyri of neocortex (posterior frontal, superior temporal, parietal and anterior occipital) exposed by craniotomy. After a 6-months survival the animals were processed either for biochemistry (Choline acetyltransferase-ChAT assay) or for immunocytochemistry (ChAT and NGF-receptor immunostaining). In lesioned/vehicle-treated monkeys the nucleus basalis of Meynert (nbM) underwent retrograde degeneration. Choline acetyltransferase (ChAT) activity significantly decreased to  $69 \pm 5\%$  of sham operated value. The morphometrical analysis revealed a significant shrinkage of ChAT immunoreactive (IR) neurons ( $61 \pm 1.4\%$  of sham operated cross-sectional area) and a decrease of fiber length ( $59 \pm 10\%$  of sham operated value) only in the intermediate nbM region. The reported decrease of ChAT activity was fully prevented with the administration of rhNGF alone or in combination with GM1. However, the shrinkage of nbM ChAT IR neurons was completely prevented only in animals receiving rhNGF in combination with GM1 ( $89 \pm 3.6\%$  of sham operated cross-sectional area). Moreover, the image analysis of fiber length showed a partial recovery of nbM neuritic processes in double treated monkeys.

These results indicate that early administration of rhNGF and rhNGF combined with GM1 significantly reduce the retrograde cell degeneration of the nbM cholinergic neurons in the cortical injured nonhuman primate. (Supported by the Medical Research Council of Canada, the Centres of Excellence Network for Neural Regeneration and Functional Recovery, Genentech Inc. and Fidia Research Labs).

#### P53 DEGENERATION AND REGENERATION OF PERIPHERAL NERVES ARE AFFECTED BY ACETYL-L-CARNITINE TREATMENT.

AM Di Giulio, A Gorio, B Tenconi, E Germani, A Bertelli, P Mantegazza, \*C De Angelis, \*C Scarfo', \*M Falcinelli, \*MT Ramacci, \*\*L Angelucci. Dept of Medical Pharmacology, Univ of Milano, Via Vanvitelli 32, 20129 Milano; \*Inst for Research on Senescence, Sigma Tau, Pomezia (Roma); \*\*Inst of Pharmacology, Medical School, Univ La Sapienza, Piazzale A. Moro 5, Roma; Italy

Lesion to peripheral nerve causes degeneration of the distal stump and, in case of permanent axotomy, also severe atrophy and retrograde degeneration of the sensory axons. Acetyl-L-carnitine (ALCAR), supplied in drinking water over the 24 hours at the dose of 150 mg/kg/day, fully counteracts the retrograde changes evidenced by degeneration of the sensory input to the spinal cord and by atrophy of the interneurons of the substantia gelatinosa. The quantitation was performed by radioimmunoassay and immunocytochemistry with specific antibodies for substance P and met-enkephalin. The morphological evaluation of regenerating sciatic nerve axons after crush injury showed that ALCAR treatment stimulates regeneration by markedly enhancing the number of regenerating axons at the early time after injury and by promoting the morphological normalization of the nerve by accelerating phagocytosis by Schwann cells of the degenerating injured axons. The positive effect of ALCAR treatment was also observed on the size of regenerated axons, that closely resemble controls with a reduced amount of small axons, that are abundant in regenerated nerves. ALCAR treatment also stimulates intramuscular sprouting of regenerating nerves. In conclusion these data suggest that ALCAR treatment prevents retrograde degeneration and promotes nerve regeneration.

**SESSION 8: TROPHIC INTERACTIONS**

**POSTER ABSTRACTS**

**P54 AXONAL TRANSPORT AND PERIPHERAL NERVE FUNCTIONS ARE ALTERED IN EXPERIMENTAL DIABETES. EFFECTS OF ACETYL-L-CARNITINE TREATMENT**

A Gorio, AM Di Giulio, B Tenconi, ML Donadoni, A Bertelli, P Mantegazza, \*F Maccari, \*L Pacifici, \*A Bellucci, \*P Piovesan, \*ML Ramacci. Dept of Medical Pharmacology, Univ of Milano, Via Vanvitelli 32, 20129 Milano; \*Institute for Research on Senescence, Sigma Tau, Pomezia (Roma); Italy

Diabetic neuropathy was induced by a single injection of alloxan (100 mg/kg). Seven days later glycemia was measured; only those animals having glycemia levels above 500 mg/dl entered the study. A group of diabetic animals received then acetyl-l-carnitine (ALCAR) at the dose of 150 mg/kg/day, dissolved in drinking water. After 5 weeks of diabetes the axonal transport of substance P (SP) was evaluated; in control rats the anterograde accumulation was seven fold above basal values and the retrograde was two fold; in diabetic rats the anterograde axonal transport was reduced by 50% and the retrograde totally abolished. ALCAR treated diabetic animals did not show reductions of SP axonal transport. In chronic diabetic rats (10 weeks following alloxan injection) the SP contents of sciatic nerve and lumbar spinal cord were significantly reduced and the nerve conduction velocity as well. ALCAR treatment of diabetic rats prevented all these alterations. This treatment also greatly reduced the extent of diabetic autonomic neuropathy of the gut, evaluated by assaying the gut innervation by SP, met-enkephalin and vasoactive intestinal polypeptide.

## SESSION 9: ELECTROPHYSIOLOGICAL ASPECTS OF CHOLINERGIC MECHANISMS

### SYMPOSIUM ABSTRACTS

- S33 INTRODUCTION: ELECTROPHYSIOLOGICAL ASPECTS OF CHOLINERGIC MECHANISMS Alexander G. Karczmar, Hines VA Hosp. and Dept. of Pharmacology, Loyola U. Med. Center, Maywood, IL 60153, USA.

Demonstration of cholinergic neurotransmission constituted first instance of the chemical transmission, i.e., chemically mediated postsynaptic response to presynaptic stimulation, first proven for parasympathetic periphery in the twenties, and for CNS in the fifties. Originally, Loewi, Feldberg, Dale, Eccles and Kibyakov envisaged this transmission as a simple, one-on-one relay process, although Dale recognized that two receptors, muscarinic and nicotinic, are involved in the postsynaptic responses. However, in the sixties Eccles described vectorial interaction between I and E currents, and subsequently it was proposed that "modulation" (Karczmar et al., 1972) provides a subtle control of transmission. A number of modulatory mechanisms were demonstrated since; thus, Krnjevic who for a number of years now considered the central muscarinic response as modulatory and facilitatory rather than synaptic in nature, proposes in this Session that changes in excitability and in synaptic function are mediated via changes in K<sup>+</sup> current. Another modulation is provided by desensitization (Thesleff, 1955) generated by a specific phosphorylation of nicotinic receptor as proposed by Greengard (Huganir et al., 1986). The reciprocal modulation is constituted by sensitization (Karczmar, 1957), the mechanism being speculatively the allosteric receptor change. At this session, McCormick and Brown emphasize the multiplicity of postsynaptic cholinergic processes; these processes differ depending on involvement and type of the G protein mediating the channel phenomena; multiple receptors families generating multiple ionic effects; several second and third messenger phenomena; membrane adaptation and recovery; and ontogenetic status of the synapses. Furthermore, initial ecclesian demonstration of I and E interaction was expanded over the years to include perhaps twenty transmitters and modulators impinging on a single postsynaptic area. Furthermore, while modulations depending on presynaptic flexibility is not a subject here, different transmitters may be released depending on presynaptic status, and presynaptic changes in release of a single transmitter engender different postsynaptic responses. Altogether total postsynaptic modulation depends on presynaptic changes, on interaction between postsynaptic responses to several transmitters and modulators, and on other processes mentioned here. Supported by CARES and NIH grants NS18710 and NS15848.

- S34 CENTRAL CHOLINERGIC MECHANISMS AND FUNCTION

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The most widespread and clearly identified actions of ACh in the brain are mediated by muscarinic receptors. Some are presynaptic (on nerve terminals) and modulate synaptic transmission - most often by diminishing transmitter release. If the synapses are excitatory, ACh reduces post-synaptic cell firing; if they are inhibitory, ACh's disinhibitory effect can lead to greatly enhanced excitation. The post-synaptic actions are predominantly (but not exclusively) excitatory. They tend to facilitate cell firing by depressing a variety of K currents: including the voltage dependent  $I_M$ ,  $I_A$ , and  $I_D$ , as well as such voltage-independent K currents as  $I_{AHP}$  (Ca-influx dependent) and a leak current. The net result thus depends on the initial state of polarization of the given cell; this determines which, if any, of these currents is already (or can be) activated. In general, the most prominent effect is to increase responses to depolarizing inputs, notably by promoting - and especially *prolonging* - repetitive discharges that normally are cut off by *afterhyperpolarizations*. By potentiating neuronal depolarization and firing, cholinergic activity has an important modulating function which would facilitate long-term ("plastic") changes in synaptic transmission. But in hypoxic/ischemic conditions, when cell firing is suppressed by the activation of K outward currents, this protective mechanism can be very effectively opposed when ACh suppresses the high anoxic K conductance. In addition, ACh tends to increase intracellular free  $[Ca^{2+}]$ . Thus, under hypoxic conditions, cholinergic activity is likely to be deleterious and may compromise long-term survival of neurons in brain regions - such as the hippocampus - that are particularly susceptible to hypoxic/ischemic damage.

## SESSION 9: ELECTROPHYSIOLOGICAL ASPECTS OF CHOLINERGIC MECHANISMS

### SYMPOSIUM ABSTRACTS

#### S35 Postsynaptic action of acetylcholine: the coupling of receptors and receptor subtypes to ion channels

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Molecular biological techniques have demonstrated the existence of at least five different genes encoding muscarinic receptors. We have carried out patch-clamp experiments in NG108-15 neuroblastoma cells which were transfected with four of these receptors (m1-m4) to determine the nature and mechanisms of coupling of muscarinic receptors to  $\text{Ca}^{2+}$  and  $\text{K}^{+}$  channels. A proportion of the high voltage-activated  $\text{I}_{\text{Ca}}$  in NG108-15s is inhibitable by neurotransmitters and m4 receptors (expressed natively) and transfected m2 receptors (but not m1 or m3 receptors) couple to inhibit this current component. Although m2 and m4 receptors also inhibit adenylyl cyclase in NG108-15 cells (via  $\text{G}_i$ ), this does not appear to be the mechanism for  $\text{I}_{\text{Ca}}$  inhibition, which (by extrapolation from studies of noradrenaline inhibition of  $\text{I}_{\text{Ca}}$ ) is probably via  $\text{G}_0$ . Transfected m1 and m3 receptors increase  $\text{Ca}^{2+}$ , by stimulating PLC to generate  $\text{IP}_3$ , thereby releasing  $\text{Ca}^{2+}$  from intracellular stores. In NG108-15s this results in activation of  $\text{I}_{\text{K(Ca)}}$  and this effect is mimicked by injection of  $\text{IP}_3$  and is blocked by  $\text{Ca}^{2+}$  buffering (eg with BAPTA). The M-type  $\text{K}^{+}$  current in NG108-15s is inhibited by stimulation of transfected m1 and m3 receptors, but not by m2 and m4 receptors. Ongoing studies to identify the second messenger and the G protein mediating this inhibition have eliminated many possibilities, but to date the nature of the linkage between m1/m3 receptors and M-current inhibition remains elusive.

This work is supported by the UK Medical Research Council.

#### S36 DISCUSSION: ELECTROPHYSIOLOGICAL ASPECTS OF CHOLINERGIC MECHANISM

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A number of different systems in the peripheral and central nervous system utilize acetylcholine as a neurotransmitter. The functional versatility of ACh is enhanced by a multiplicity of cholinergic receptors and actions. There exist at least four different alpha subunits for nicotinic receptors which take part in the formation of nicotinic channels and five different subtypes of muscarinic receptors (m1-m5) coupled to various G-proteins. Examination of cholinergic electrophysiological actions have demonstrated two major classes of postsynaptic response: rapid depolarization (msecs) through nicotinic receptors via opening of cation channels and slow (100's of msecs to seconds) excitation or inhibition through muscarinic receptors through decreases or increases in various  $\text{K}^{+}$  currents, or the suppression of  $\text{Ca}^{++}$  currents. The  $\text{K}^{+}$  currents which are reduced by acetylcholine can either be voltage independent, voltage dependent (M-current) or  $\text{Ca}^{++}$ -activated ( $\text{I}_{\text{AHP}}$ ). Block of a voltage independent  $\text{K}^{+}$  current results in an overall increase in responsiveness of the neuron to phasic synaptic inputs and a depolarization towards firing threshold. Decreases in  $\text{I}_{\text{M}}$  and  $\text{I}_{\text{AHP}}$ , on the other hand, preferably enhance the response of the neuron to depolarizing trains of EPSPs and prolonged depolarization. Muscarinic receptor mediated increases in  $\text{K}^{+}$  current presumably occur through cellular mechanisms similar to those in the heart. Current questions center on the identification of the various subtypes of receptors which mediated the different ionic responses, the intracellular events which couple these receptors to their ionic responses, and the functional implications of these responses on the activity of neurons and neuronal circuits.



## SESSION 9: ELECTROPHYSIOLOGICAL ASPECTS OF CHOLINERGIC MECHANISMS

### POSTER ABSTRACTS

- P55 **ELECTROPHYSIOLOGY OF AN EXCITATORY NICOTINIC SYNAPSE IN THE CENTRAL NERVOUS SYSTEM.** V.A. Chiappinelli, W.R. Weaver and L.L. McMahon. Dept. of Pharmacological and Physiological Science, Saint Louis University School of Medicine, St. Louis MO 63104, USA.

Neurons in the mesencephalic lateral spiriform nucleus (SPL) of the newly hatched chick respond to nicotine with a marked depolarization and increased excitability. The nicotinic receptors in this nucleus have a high affinity for  $^3\text{H}$ -nicotine, are insensitive to blockade by kappa-bungarotoxin or  $\alpha$ -bungarotoxin (Sorenson and Chiappinelli, *Neuron* 5:307, 1990), and are likely to consist of  $\alpha 2$  and/or  $\alpha 4$  subunits in combination with  $\beta 2$  subunits (Morris et al., *Mol. Brain Res.* 7:305, 1990). Using intracellular recording in brain slices as previously described (Sorenson and Chiappinelli, *ibid*), we have now established that these nicotinic receptors mediate subthreshold and suprathreshold excitatory postsynaptic potentials (EPSPs) in response to stimulation of a cholinergic nerve tract (Sorenson et al., *J. Comp. Neurol.* 281:641, 1989) located laterally to the SPL. All EPSPs were blocked in the presence of the calcium channel blocker  $\text{Cd}^{+2}$  (100  $\mu\text{M}$ ). The synaptic delay at this central nicotinic synapse is 3-5 msec. Nicotinic antagonists such as d-tubocurarine (50  $\mu\text{M}$ ) and dihydro- $\beta$ -erythroidine (100  $\mu\text{M}$ ) blocked both evoked EPSPs and responses to exogenous nicotinic agonists at comparable concentrations. Whole cell patch clamp records of SPL neurons in brain slices reveal the presence of inward miniature excitatory postsynaptic currents that are due to the spontaneous release of acetylcholine at this synapse. The chick SPL is thus the second example of an excitatory nicotinic synapse in the central nervous system. The first such synapse to be described is located in the spinal cord, and mediates transmission between motor neurons and Renshaw cells (Curtis and Eccles, *J. Physiol.* 141:446, 1958). Thus, while many central nicotinic receptors appear to be presynaptic in nature, at least some postsynaptic nicotinic receptors exist within the brain, where they function much like those at the Renshaw synapse or in autonomic ganglia. Supported by NIH Grant NS17574 to V.A.C.

- P56 **MUSCARINIC AND METABOTROPIC (GLUTAMATE) RECEPTOR AGONISTS INDUCE A SIMILAR POST-STIMULUS AFTERDEPOLARIZATION IN GUINEA-PIG OLFACTORY CORTEX NEURONES IN VITRO.** A. Constanti and V. Libri. Dept. of Pharmacology, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX.

'Metabotropic' glutamate receptors are, like muscarinic acetylcholine receptors, linked to inositol phospholipid hydrolysis (Schoepp et al., 1991, *J. Neurochem.* 56, 1789:). These receptors are selectively activated by trans ( $\pm$ )-1-amino-1,3, cyclopentanedicarboxylic acid (trans-ACPD). Activation of metabotropic receptors on hippocampal neurones causes a slow depolarization and suppression of  $\text{K}^+$  conductances (Chrapak et al., 1990, *Nature*, 347, 765:), effects very similar to those induced by muscarinic activation. In olfactory cortical neurones, muscarinic agonists produce a *persistent* excitation and appearance of a prolonged post-stimulus inward tail current ( $I_{\text{ADP}}$ ; Constanti & Bagetta, 1991, *Neurosci. Letts.* 131, 27:). We examined whether trans-ACPD could induce a similar tail current in cortical neurones studied intracellularly using guinea-pig olfactory cortex slices maintained at 28-30°C. In 11/17 cells, trans-ACPD (50  $\mu\text{M}$ , 1-2 min) produced a slow depolarization (mean  $\pm$  s.e.m. =  $7 \pm 0.5$  mV) up to firing threshold, a small increase in input resistance ( $15 \pm 3$  %) and a sustained neuronal firing that persisted for up to 20 min following washout. In trans-ACPD, a slow after-depolarization (sADP) appeared following a 1-5s depolarizing stimulus; under voltage clamp, the underlying slow  $I_{\text{ADP}}$  resembled the  $\text{K}^+$ -mediated  $I_{\text{ADP}}$  induced by muscarinic agonists, but was insensitive to atropine (500 nM-1  $\mu\text{M}$ ).  $I_{\text{ADP}}$  was reduced by  $\text{Cd}^{2+}$  (100  $\mu\text{M}$ ), tetrabutylammonium (500  $\mu\text{M}$ ) or by amiloride (1 mM). Activation of metabotropic or muscarinic receptors on olfactory neurones can thus induce an  $I_{\text{ADP}}$  tail current that contributes to long-term changes in neuronal excitability. A common intracellular transduction mechanism might be responsible for  $I_{\text{ADP}}$  generation in these cells. Supported by the Wellcome Trust.

## SESSION 9: ELECTROPHYSIOLOGICAL ASPECTS OF CHOLINERGIC MECHANISMS

### POSTER ABSTRACTS

- P57 **ROLE OF TARGET TISSUES IN THE SWITCH OVER OF  $Ca^{++}$  CHANNELS COUPLED TO ACh RELEASE DURING DEVELOPMENT.** Pilar, G., Gray, D.B., Rossi, C., and Korn, S. Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06269.

It is known that many properties of synaptic transmission change during development, but little is known about changes in the coupling of  $Ca^{++}$  channels and ACh release. The specificity of  $Ca^{++}$  channel secretion coupling was studied in the chick eye choroid neuromuscular junctions and in dissociated ciliary ganglion (CG) neurons cocultured with or without muscles. ACh secretion changes in development from stage (St) 40, when release is dihydropyridine (DHP) and partially  $\omega$ -conotoxin ( $\omega$ -CgTX) sensitive, to posthatch, when release is insensitive to DHPs but sensitive to  $\omega$ -CgTX. St 40 CG neurons cultured with striated muscle have release properties similar to those of St 40 iris and choroid. At embryonic day 14 evoked ACh release from CG neurons cocultured with muscle is mediated by calcium influx through channels which are inhibited by nifedipine. In cultures of CG neurons alone, this release is not sensitive to nifedipine. This dependence on the presence of muscle does not appear to be mediated by soluble trophic factors released by the muscle. Neurons cultured alone were either exposed to media conditioned by muscle or grown on aclar cover slips to prevent contact between neurons and muscle, and placed in muscle cultures for 24 hours. Nifedipine was unable to inhibit evoked release in either condition. However, evoked release from St 40 CG neurons grown on lysed myotube membranes for 24 hrs was reduced by 50% ( $p < .05$ ) by  $10\mu M$  nifedipine. These results suggest that contact of neurons with muscle cell membrane can induce the coupling of L-like channels to ACh secretion. We are currently examining the pharmacology of whole cell calcium currents to determine if changes in the coupling of channels to secretion observed above may be mediated by differential expression of calcium channels in these cells. Supported by NIH grant NS 10338, the Whittaker Foundation and The University of Connecticut Research Foundation.

- P58 **ACTIONS OF VERATRIDINE ON SPONTANEOUS QUANTAL ACh RELEASE AT THE RAT NEUROMUSCULAR JUNCTION.**

K.E. Pemberton, C. Prior and I.G. Marshall. Dept. Physiol. and Pharmacol., University of Strathclyde, Glasgow, U.K.

We have studied the effects of veratridine (a sodium channel activator) on spontaneously released acetylcholine (ACh), measured as miniature end-plate currents (MEPCs), using the cut rat hemidiaphragm ( $32^{\circ}C$ , voltage clamped at  $-55mV$ ). MEPCs were recorded for 2 min. before applying veratridine for 4 min. Following washout of veratridine a further recording of MEPCs was taken. Post-veratridine MEPCs were analysed over a 2 min. period taken 1 min. after initiation of washout. Incubation in  $40\mu M$  veratridine initially resulted in a large increase in the frequency of the MEPCs. Following application of the veratridine there was a significant reduction in the overall mean MEPC amplitude and a large increase in the distribution of MEPC amplitudes. The post-veratridine MEPC amplitude frequency histograms were well fitted by a double Gaussian function comprising two discrete populations of MEPCs. The mean amplitude of the larger MEPCs was  $86.6 \pm 2.8\%$  ( $n=5$ ) of pre-veratridine control, and the mean amplitude of the smaller MEPCs was  $40.0 \pm 4.4\%$  of control. These results show a remarkable similarity to those seen after short periods of high frequency nerve stimulation in the presence of a sub-maximal dose of the synaptic vesicle ACh-transport inhibitor, (-)-vesamicol (Searl *et al.*, 1991: *J. Physiol.* 144, 99-116). Exposure to  $40\mu M$  veratridine for 4 min. in the presence of  $1\mu M$  (-)-vesamicol (a concentration maximal for its inhibitory effects on vesicular ACh uptake) resulted in a single population of MEPCs that was well fitted by a single Gaussian curve and was uniformly reduced in mean amplitude ( $77.6 \pm 2.1\%$  of control,  $n=5$ ). The sensitivity of the smaller amplitude population of MEPCs to (-)-vesamicol suggests that it represents recycling quanta and that, in addition to its sodium channel effects, veratridine may be having an inhibitory effect on ACh uptake. The reduction in mean amplitude of the larger amplitude population of MEPCs is probably due to a post-junctional effect of veratridine.

The research was funded by a Wellcome Trust project grant. KP holds an MRC postgraduate studentship.

## SESSION 9: ELECTROPHYSIOLOGICAL ASPECTS OF CHOLINERGIC MECHANISMS

### POSTER ABSTRACTS

#### P59 THE EFFECTS OF AMMONIUM IONS ON MINIATURE END-PLATE CURRENT AMPLITUDE DISTRIBUTIONS AT THE SNAKE NEUROMUSCULAR JUNCTION.

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We have previously shown that, at the snake neuromuscular junction, periods of prolonged nerve stimulation lead to the appearance of an additional population of miniature end-plate currents (MEPCs) with a mean amplitude of approximately one-half of the normal-sized events (Searl *et al.*, 1990: *Neuroscience* 35, 145-156). Based on their sensitivity to (-)-vesamicol, an inhibitor of the synaptic vesicle ACh-transporter, we have attributed these small-mode MEPCs to the release of recycling quanta generated as a consequence of the period of sustained transmitter release. It has been established, in isolated test systems, that the concentrative uptake of ACh by synaptic vesicles utilizes a trans-vesicular membrane proton gradient (internal acidic), established by a vesicular membrane proton-pumping ATPase. To determine the role of this proton gradient in the formation of recycling quanta we have now studied the effects of ammonium ions, known to be able to dissipate trans-membrane proton gradients, on the stimulation-induced appearance of small-mode MEPCs in the snake. MEPCs were recorded at -110 mV from end-plates in cut muscle fibres of the snake costocutaneous muscle for 4 min. before and after a 5 min. period of 10 Hz nerve stimulation. All experiments were performed in the presence of 10 mM  $\text{NH}_4\text{Cl}$ . In direct contrast to results seen in the absence of ammonium ions, 10 Hz nerve stimulation for 5 min. produced no change in either the mean or coefficient of variance (c.v.) of MEPC amplitudes. Thus the pre- and post-stimulation mean MEPC amplitudes were both  $3.4 \pm 0.2$  nA ( $n=6$ ) and the pre- and post-stimulation c.v. of MEPC amplitudes were  $19.4 \pm 0.8$  % and  $21.7 \pm 0.7$  % ( $n=6$ ) respectively. Also both pre- and post-stimulation MEPC amplitude frequency histograms were well fitted by similar single Gaussian functions. Thus ammonium ions appear capable of inhibiting the stimulation-induced appearance of small-mode MEPCs. The absence of the small-mode MEPCs indicates a total block of vesicle recycling and strongly suggests a functional role for the trans-vesicular membrane proton gradient in the formation and filling of recycling quanta.

Supported by project grants from the Wellcome Trust.

#### P60 ANALYSIS OF (-)-VESAMICOL-INDUCED RUNDOWN OF END-PLATE CURRENT AMPLITUDES IN THE RAT.

L. Tian, C. Prior, J. Dempster & I.G. Marshall. Dept. Physiol. & Pharmacol., University of Strathclyde, Glasgow, U.K.

Vesamicol inhibits the uptake of acetylcholine (ACh) by synaptic vesicles within cholinergic nerve terminals. Also, the compound increases the rundown of the amplitude of successive end-plate currents (EPC) during high frequency trains of nerve stimulation. This latter action reflects a reduction of transmitter mobilization by the compound (Pemberton *et al.*, 1992: *Br. J. Pharmacol.* 105, 113-118). The mechanism underlying this effect of vesamicol is unknown. This present study uses binomial statistical analysis of EPC amplitudes (Miyamoto, 1975: *J. Physiol.* 245, 447-466) to determine the change in quantal release parameters ( $n$ ,  $p$  and quantal content) associated with (-)-vesamicol-induced rundown of EPC amplitudes at high rates of nerve stimulation. Miniature end-plate currents (MEPCs) and EPCs were recorded from the cut rat hemidiaphragm at 32°C using a two-electrode voltage clamp technique (holding potential, -50mV). The motor nerve was stimulated at 50 Hz for 2 s and release parameters were calculated from the amplitude of the last 80 EPCs. Vesamicol (1  $\mu\text{M}$ ) did not affect either the quantal content of the first EPC in the train ( $\text{EPC}_1$ ) or the mean MEPC amplitude.  $\text{EPC}_1$  quantal content: control,  $67.9 \pm 8.3$ ; vesamicol,  $70.2 \pm 7.8$  ( $n=7$ ,  $P>0.05$ , paired Student's  $t$  test). MEPC amplitude: control,  $1.9 \pm 0.1$  nA; vesamicol,  $2.0 \pm 0.1$  nA ( $P>0.05$ ). However, 1  $\mu\text{M}$  (-)-vesamicol increased EPC amplitude rundown from  $25.3 \pm 3.0$  % in control to  $36.1 \pm 2.9$  % ( $P<0.05$ ). Binomial analysis shows that the increased EPC amplitude rundown reflects a decreased quantal content towards the end of the stimulation burst in the presence of vesamicol. Thus quantal content of the last 80 EPCs was reduced from  $50.7 \pm 6.4$  in control to  $45.3 \pm 6.0$  in 1  $\mu\text{M}$  (-)-vesamicol ( $P<0.05$ ). This was due to a reduction in the size of the immediately available pool of quanta ( $n$ ): control,  $68.2 \pm 9.8$ ; vesamicol,  $59.8 \pm 9.5$  ( $P<0.05$ ). Probability of release ( $p$ ) remained unchanged: control,  $0.77 \pm 0.07$ ; vesamicol,  $0.78 \pm 0.05$  ( $P>0.05$ ). We therefore conclude that the principle effect of vesamicol on ACh mobilization is to reduce quantal content by limiting the supply of synaptic vesicles for release rather than affecting the probability of release.

Funded by project grants from the Wellcome Trust.

## SESSION 10: SECOND MESSENGERS

### SYMPOSIUM ABSTRACTS

#### S37 $\text{Li}^+$ INCREASES ACCUMULATION OF INOSITOL 1,4,5-TRISPHOSPHATE AND INOSITOL 1,3,4,5-TETRAKISPHOSPHATE IN CHOLINERGICALLY STIMULATED BRAIN CORTEX SLICES IN MOUSE, RAT AND GUINEA PIG AND OF INOSITOL 1,4,5-TRISPHOSPHATE IN RHESUS MONKEY.

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$\text{Li}^+$ , beginning at a concentration as low as 1 mM, produced a time- and dose-dependent increase in accumulation of [ $^3\text{H}$ ]inositol 1,4,5-trisphosphate [ $\text{Ins}(1,4,5)\text{P}_3$ ] and [ $^3\text{H}$ ]inositol 1,3,4,5-tetrakisphosphate [ $\text{Ins}(1,3,4,5)\text{P}_4$ ] in ACh-stimulated guinea pig brain cortex slices prelabelled with [ $^3\text{H}$ ]inositol. Similar results were obtained by mass measurement of samples incubated with  $\text{Li}^+$  using a receptor binding assay. The increase in accumulation of  $\text{Ins}(1,4,5)\text{P}_3$  and  $\text{Ins}(1,3,4,5)\text{P}_4$  by  $\text{Li}^+$  was absolutely dependent on the presence of ACh. In previous studies with cholinergically stimulated rat and mouse brain cortex slices,  $\text{Li}^+$  inhibited or produced no change in accumulation of  $\text{Ins}(1,4,5)\text{P}_3$  and inhibited  $\text{Ins}(1,3,4,5)\text{P}_4$  accumulation [Batty, I.R. & Nahorski, S.R. (1987) *Biochem. J.* 247, 797-800; Whitworth, P. & Kendall, D.A. (1988) *J. Neurochem.* 51, 258-265]. We found that  $\text{Li}^+$  inhibited both  $\text{Ins}(1,4,5)\text{P}_3$  and  $\text{Ins}(1,3,4,5)\text{P}_4$  accumulation in these species, but we could reverse this inhibition by adding 10-30 mM inositol; we then observed a  $\text{Li}^+$ -induced increase in levels of  $\text{Ins}(1,4,5)\text{P}_3$  and  $\text{Ins}(1,3,4,5)\text{P}_4$  in these species also. These data suggest that inositol is more rate-limiting for PI synthesis in the presence of  $\text{Li}^+$  in rat and mouse, which can account for the previous reports of no increase or inhibition in  $\text{Ins}(1,4,5)\text{P}_3$  and  $\text{Ins}(1,3,4,5)\text{P}_4$  accumulation by this ion in these species. With regard to the therapeutic effects of  $\text{Li}^+$  in man, an animal model as close to man as possible is desirable. We have recently been studying the effects of  $\text{Li}^+$  on levels of  $\text{Ins}(1,4,5)\text{P}_3$  and  $\text{Ins}(1,3,4,5)\text{P}_4$  in brain cortex slices from rhesus monkeys. 1 mM  $\text{Li}^+$  increased levels of  $\text{Ins}(1,4,5)\text{P}_3$  substantially in this species, but concentrations of  $\text{Li}^+$  as high as 5 mM had no effect on  $\text{Ins}(1,3,4,5)\text{P}_4$  levels. Supplementation with inositol was not required. Interestingly, no agonist was required to show the  $\text{Li}^+$  effect. Also of interest was the finding that of a variety of neurotransmitter antagonists only antagonists to the NMDA receptor inhibited the  $\text{Li}^+$  effect, although there was a marginal effect of atropine. These observations will be discussed *vis-a-vis* the mechanism of action of  $\text{Li}^+$  in the treatment of bipolar disorders.

#### S38 LITHIUM SELECTIVELY POTENTIATES CHOLINERGIC ACTIVITY IN RAT BRAIN.

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Lithium is the primary drug used to treat manic-depressive disorders, but its mechanism of action remains unknown. Because cholinergic activity may be reduced in mania, we tested the hypothesis that lithium selectively enhances cholinergic activity. This hypothesis was supported by several findings. (1) EEG recordings were used to show that therapeutic levels of lithium after acute (3 mmol/kg) or chronic (4 weeks, dietary) administration to rats enhanced CNS responses to cholinergic agonists (pilocarpine, arecoline, physostigmine) given peripherally. One clear measure that we have used of the potentiated responses is the induction of seizures by administration of lithium and pilocarpine. (2) Lithium plus pilocarpine caused 5-fold increases in cortical ACh concentration and increased ACh release. (3) The early phase of potentiation causing the initiation of seizures is blocked by atropine but once seizures begin NMDA receptor activation is responsible for their maintenance. Thus, the NMDA antagonist MK-801 did not block the first stages of seizures but it blocked the development of status epilepticus. This indicates that cholinergic stimulation led to recruitment of excitatory amino acid pathways. (4) Lithium specifically enhances responses to cholinomimetics; it does not potentiate the responses to other convulsants. (5) Pertussis toxin, which inactivates some G-proteins, mimics the cholinomimetic-enhancing effect of lithium, supporting the hypothesis that lithium may interfere with G-protein-mediated processes. (6) Lithium plus pilocarpine selectively increases the tyrosine phosphorylation of a 40 kD protein. Results from other laboratories indicates that this 40 kD protein is probably the important regulatory enzyme, MAP kinase. In conclusion, this series of experiments has demonstrated that lithium selectively enhances cholinergic activity in rat brain.

## SESSION 10: SECOND MESSENGERS

### SYMPOSIUM ABSTRACTS

#### S39 CHARACTERISTICS OF THE CHANGES IN THE CYTOSOLIC $\text{Ca}^{2+}$ CONCENTRATION DURING THE STIMULATION OF MUSCARINIC RECEPTOR IN HIPPOCAMPAL NEURONS

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Changes in cytosolic free  $\text{Ca}^{2+}$  concentration ( $[\text{Ca}^{2+}]_i$ ) in response to acetylcholine (ACh) were examined by fura-2 fluorometry in rat hippocampal neurons. ACh caused various patterns of change in the  $[\text{Ca}^{2+}]_i$  in cultured hippocampal neurons. In most cases,  $[\text{Ca}^{2+}]_i$  increase was accompanied by a membrane depolarization detected by a whole cell patch electrode. The change in  $[\text{Ca}^{2+}]_i$  was significantly reduced when the membrane potential was clamped at -55 mV indicating the participation of voltage operated  $\text{Ca}^{2+}$  channels in the change. We found two types of depolarization associated with the decrease and increase in membrane conductance. Since a part of the increase in  $[\text{Ca}^{2+}]_i$  was detected even in  $\text{Ca}^{2+}$ -free medium, the receptor coupled with G-protein was suggested to be involved in stimulating intracellular  $\text{Ca}^{2+}$  store site. Furthermore we found increase in  $[\text{Ca}^{2+}]_i$  associated with hyperpolarization which may be the result of the stimulation of  $\text{Ca}^{2+}$  dependent  $\text{K}^+$  channel through the  $\text{Ca}^{2+}$  released from the intracellular store site. These responses were confirmed to be muscarinic by pharmacological studies. The regional distribution of the ACh receptors concerning to the increase in  $[\text{Ca}^{2+}]_i$  in hippocampus were examined in fresh slice and also slice culture preparations loaded with fura-2. We found in this study the density of the distribution of ACh receptor in the hippocampus was higher in CA1 synaptic region. The possible contribution of cholinergic receptors to the synaptic modulation through the change in  $[\text{Ca}^{2+}]_i$  will be discussed.

#### S40 MUSCARINIC RECEPTOR SUBTYPE SELECTIVITY FOR NEURONAL SECOND MESSENGER SYSTEMS. M. McKinney, Mayo Clinic Jacksonville, Jacksonville, FL 32224.

The cloned muscarinic receptors (m1-m5) display relatively selective, G-protein-dependent, coupling to two major classes of biochemical second messenger systems: cyclic AMP inhibition (m2, m4) and phosphoinositide (PI) metabolism (m1, m3, m5). Additionally, muscarinic receptors are known to activate other phospholipases and potassium channels. The inhibition of cyclic AMP usually results from a direct effect on the Gi/Go subclasses of G-proteins but cyclic AMP levels also may be reduced by calcium-dependent activation of phosphodiesterase. The PI transduction system intersects with mechanisms of regulation of intracellular calcium (via IP3), regulation of ion channels (via IP3/DAG-mediated PKC action and/or the action of calcium), regulation of intracellular enzymes-including those in other transduction systems (via calcium/calmodulin and/or phosphorylation by PKC), as well as other "downstream processes" such as neurotransmitter release and transcription. Since these manifold intracellular systems can potentially interact, it is not always straightforward to predict which muscarinic receptor subtype underlies a given cellular effect. We have applied several pharmacological techniques to study the coupling of neuronal muscarinic receptors to the regulation of cyclic AMP levels, PI metabolism, and acetylcholine release. Our findings indicate that cortical and hippocampal PI turnover involves the M1 and M3 receptors, while M4 receptors couple to cyclic AMP inhibition (cortex and striatum) and to the inhibition of acetylcholine release in hippocampus. Cyclic GMP elevation in response to muscarinic M1 stimulation appears to involve the novel second messenger nitric oxide, probably in response to elevated cytosolic calcium ion.

SESSION 10: SECOND MESSENGERS

POSTER ABSTRACTS

- P61 **ABNORMAL ACTIVITY OF NICOTINIC ACETYLCHOLINE RECEPTOR CHANNELS IN MYOTUBES FROM *dy* MICE** Alfredo Franco-Obrégon and Jeffry B. Lansman.  
Department of Pharmacology, UCSF, San Francisco, CA. 94143.

The activity of nicotinic acetylcholine receptor channels recorded from cell-attached patches from *dy* myotubes differs from that recorded from wild type myotubes in two ways. First, in the absence of acetylcholine added to the patch electrode, spontaneous openings of acetylcholine receptor channels are observed at a much greater frequency in *dy* myotubes. Secondly, the sensitivity of acetylcholine receptor channels to acetylcholine appears to be reduced in *dy* myotubes when compared to wild type myotubes. Excising patches of myotube membrane from the surface of *dy* myotubes results in a steady drop in spontaneous activity, suggesting that diffusible cytosolic second messengers are responsible for maintaining spontaneous openings. Adding bromo-cAMP or forskolin to cultures of wild type myotubes results in an increase of spontaneous channel openings and a concomitant reduction in the responsiveness of acetylcholine receptor channels to acetylcholine, mimicking the *dy* dystrophic condition. We propose that the *dy* phenotype could arise as a result of a defect of the adenylate cyclase signalling pathway.

- P62 **ACETYLCHOLINE-EVOKED CURRENT INDUCES  $\text{Ca}^{2+}$  RELEASE FROM  $\text{InsP}_3$ -SENSITIVE STORES IN MOUSE MYOTUBES.**  
F. Grassi, A. Giovannelli, S. Fucile, S. Cerasi, E. Mattei, F. Di Modugno, and F. Eusebi.  
Lab. of Biophysics, IRE, via delle Messi d'Oro 156, I-00158, Rome. Italy.

The metabolic consequences of the stimulation of the nicotinic acetylcholine (ACh) receptor (nAChR) were studied in cultured mouse C2 myotubes, using digital  $\text{Ca}^{2+}$  imaging fluorescence microscopy and standard HPLC techniques to detect formation of inositol (1,4,5)-trisphosphate ( $\text{InsP}_3$ ). In the absence of extracellular  $\text{Ca}^{2+}$ , cholinergic stimulation by ACh or nicotine (Nic), but not by muscarine, raises  $[\text{Ca}^{2+}]_i$  by about 10-folds, and induces the accumulation of  $\text{InsP}_3$ , with similar time courses. These two events appear to be linked by the observation that thapsigargin and neomycin, known to deplete  $\text{InsP}_3$ -sensitive  $\text{Ca}^{2+}$  stores, inhibit ACh- and Nic-induced increase of  $[\text{Ca}^{2+}]_i$ , while caffeine, which depletes the ryanodine-sensitive  $\text{Ca}^{2+}$  store, does not. In addition, the increase of  $[\text{Ca}^{2+}]_i$  followed a Poisson's distribution, in agreement with the hypothesized quantal release of  $\text{Ca}^{2+}$  from  $\text{InsP}_3$ -sensitive stores. Any change to the extracellular medium which reduces or suppresses the current through the nAChR also abolishes  $\text{Ca}^{2+}$  mobilization and  $\text{InsP}_3$  accumulation. For example, the increase of  $[\text{Ca}^{2+}]_i$  is reversibly prevented by myotube depolarization with 50 mM KCl, and by the substitution, partial or total, of extracellular  $\text{Na}^+$  with sucrose or N-methyl-D-glucamine. Under the same ionic conditions, ACh fails to significantly stimulate  $\text{InsP}_3$  accumulation. These data suggest that the current flowing through the pore of the activated nAChR alters the ionic composition of the cytosol in such a way that basal phosphoinositide turnover is stimulated, resulting in  $\text{InsP}_3$  accumulation and in the consequent release of  $\text{Ca}^{2+}$  from intracellular stores. It is at present unclear whether this increase of  $[\text{Ca}^{2+}]_i$  is relevant to muscle contraction, but it is very likely that, together with other events of the phosphoinositides turnover cascade such as activation of protein kinase C, it plays an important role in the regulation of nAChR function. This work was partially supported by a FIDIA grant (to F.E.).

## SESSION 10: SECOND MESSENGERS

### POSTER ABSTRACTS

#### P63 MOLECULAR MECHANISMS UNDERLYING THE REGULATORY EFFECTS OF NICOTINIC RECEPTOR TOWARDS MUSCARINIC RECEPTOR-EFFECTOR SYSTEMS \*

Chuan-Gui LIU, Hai WANG and WEN-Yu CUI

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The results of our experiments indicated that the facilitation of nicotinic receptor desensitization towards the sensitivity of muscarinic receptor to its agonists has been observed in parasympathetic and central nervous systems. In this experiment, we examine the regulatory effects of nicotine on muscarinic receptor-effector systems. 1. Adenylate cyclase (AC) systems: The basic activity of AC coupled with  $M_2$  and  $M_4$  receptors of rat hearts, striata and NG108-15 cell line was not influenced by incubation with nicotine (1.0nM-1.0mM). The stimulatory effects of AC activators sodium fluoride and forskoline were not influenced by nicotine (1.0nM-1.0mM) either. But the inhibitory effects of muscarinic agonist carbachol (1.0nM-0.1mM) were enhanced by nicotine 1.0 $\mu$ M, which could be antagonized by N-antagonist mecamlamine 10.0nM. It is indicated that the relationship between  $M_2$  and  $M_4$  receptor and AC were facilitated by nicotine incubation, while nicotine had no direct effects on AC activity. 2. Phosphatidylinositol (PI) turnover systems: In acute and chronic experiments, repeated administration of nicotine (0.5, 1.0, 1.0, 2.0, 2.0 mg/kg ip at 5 min intervals) could stimulate PI turnover in rat submaxillary gland, cerebral cortex and hippocampus, rather than striatum. There existed synergisms between nicotine's and arecoline's effects for stimulating PI turnover, which could be antagonized by mecamlamine 1.0mg/kg ip. It is indicated that the relationship between  $M_1$  and  $M_3$  receptors and their effector systems were also facilitated by repeated nicotine pretreatment, and nicotine could directly stimulate PI turnover. 3. Intracellular  $Ca^{2+}$  of brain neurons: Using  $^{45}Ca^{2+}$  and Fura-2 as indicators, brain nicotinic receptor activation by nicotine (0.1-10.0 $\mu$ M) induced influx of  $^{45}Ca^{2+}$  into neurons, resulting in subsequent increases of intracellular  $Ca^{2+}$  concentration by 30%, which facilitated the sensitivity of 1,4,5-inositol triphosphate ( $IP_3$ ) receptor of endoplasmic reticulum to  $IP_3$  for increasing intracellular  $Ca^{2+}$  concentration. It is suggested that in CNS and periphery nicotine can facilitate the coupling of muscarinic receptor to its effector systems.

\* This project is supported by National Natural Science Foundation of China.

#### P64 MOLECULAR MECHANISMS UNDERLYING THE REGULATORY EFFECTS OF BRAIN NICOTINIC RECEPTOR TOWARDS MUSCARINIC REOPTOR AND GTP-BINDING PROTEINS \*

Chuan-Gui LIU, Hai WANG and Wen-Yu CUI

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It has been reported by our lab that brain nicotinic receptor desensitization as a result of nicotine pretreatment selectively increases the sensitivity of brain muscarinic receptor to its agonists. In this paper we report the regulatory effects of nicotine on brain muscarinic receptor, its GTP-binding protein (G-proteins) and the coupling relationship between them. 1. Brain muscarinic receptors: Membrane fractions of rat cerebral cortex were preincubated with nicotine (0.1nM-1.0mM). The  $K_d$  values of muscarinic receptors bound with its antagonist [ $^3H$ ] QNB were concentration-dependently increased. These effects were antagonized by N-antagonist mecamylamine (10.0nM), while were not influenced by DTT (10.0 $\mu$ M). In the presence of nicotine (1.0 $\mu$ M), the rates of association and dissociation of [ $^3H$ ] QNB binding with muscarinic receptor were decreased. In the presence of nicotine 1.0 $\mu$ M, the  $K_d$  values of rat cerebral muscarinic receptor bound with its agonist [ $^3H$ ] oxotremorine-M were decreased. 2. G-proteins: Solubilized G-proteins of rats cerebral cortex was preincubated with nicotine (1.0 $\mu$ M) for 10min at 25 $^{\circ}C$ , the rates of G-proteins bound with [ $^{32}S$ ] GTP $\gamma$ S were decreased, while the binding capacity remained unchanged. 3. Coupling relationship between brain muscarinic receptor and G-proteins: Solubilized muscarinic receptor and G-protein complex of rat cerebral cortex was preincubated with nicotine (1.0 $\mu$ M) for 10min at 35 $^{\circ}C$ , the inhibitory effects of GTP $\gamma$ S on [ $^3H$ ] oxotremorine-M binding were potentiated. It is suggested that the binding properties of brain muscarinic receptor and G-proteins with its ligands and GTP $\gamma$ S were changed, and the coupling relationship between them was potentiated by the preincubation with nicotine, which may result in brain nicotinic receptor activation and subsequent desensitization.

\* This project is supported by National Natural Science Foundation of China.

## SESSION 10: SECOND MESSENGERS

### POSTER ABSTRACTS

**P65 EVIDENCE FOR THE REGULATORY EFFECTS OF DESENSITIZED GANGLIONIC NICOTINIC RECEPTOR TOWARDS MUSCARINIC RECEPTOR IN HEART, ILEUM AND SUBMAXILLARY GLAND \***

Chuan-Gui LIU, Hai WANG and Wen-Yu CUI

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It has been reported by our lab that brain nicotinic receptor desensitization as a result of nicotine pretreatment selectively increases the sensitivity of brain muscarinic receptor to its agonists. In this study we want to know whether this phenomenon is also observed in periphery. In isolated ileum of guinea-pig, ACh-induced contraction could be characterized by two phases. Excitation of parasympathetic ganglionic nicotinic receptor and smooth muscle muscarinic receptor were responsible for the initial fast contraction of short period and the subsequent slow one of long period respectively. Preincubation of isolated ileum with 10.0  $\mu$ M nicotine resulted in blockade of nicotinic effect for producing initial short contraction and enhancement of muscarinic effect for producing long-lasting slow contraction in response to the same concentration of ACh. In acute nicotine-tolerant rats (pretreated with one dose of nicotine 0.5 mg/kg iv), M-agonist carbachol produced more prominent bradycardia than that in naive rats. In acute nicotine-tolerant mice (pretreated with two doses of nicotine 0.5 and 1.0 mg/kg iv at 5 min interval) the dose-response curves of M-agonists arecoline and oxotremorine for producing salivation shifted leftward. Above demonstrated phenomena could be prevented by N-antagonist mecamylamine. The results show that parasympathetic ganglionic nicotinic receptor desensitization as a result of nicotine pretreatment increases the sensitivity of muscarinic receptors in heart, ileum and submaxillary gland to M-agonists. It is suggested that increased sensitivity of muscarinic receptor to its agonists after the desensitization of ganglionic nicotinic receptor is also observed in periphery.

\* This project is supported by National Natural Science Foundation of China.

**P66 MUSCARINIC RECEPTORS EXPRESSED ON OLIGODENDROCYTES MEDIATE PHOSPHOINOSITIDE HYDROLYSIS AND INTRACELLULAR CALCIUM MOBILIZATION.**

Cohen, R.I., Almazan, G., McGill Univ., Dept. of Pharmacology, Montreal, PQ H3G 1Y6

The presence of muscarinic acetylcholine receptors (mAChR) in purified myelin (Larocca et al., J. Neurosci. 7:3863, 1987) suggests that oligodendrocyte development is influenced by the neurotransmitter acetylcholine. To understand the functional significance of mAChR, we have characterized some of the physiological responses of oligodendrocyte precursor primary culture (OP) to muscarinic receptors agonists and antagonists. Preliminary binding studies with OP's demonstrate specific binding for [ $^3$ H]-QNB, a high affinity muscarinic antagonist. Carbachol, a cholinergic agonist, causes a fast dose-dependent increase in intracellular calcium ( $[Ca^{2+}]_i$ ), which is inhibited by the muscarinic receptor antagonist atropine. The increase in  $[Ca^{2+}]_i$  is biphasic; a convex increase, followed by a sustained plateau. The initial increase represents mobilization of  $Ca^{2+}$  from intracellular stores and the second phase, because it alone can be abolished by incubation with 1-2 mM EGTA, a  $Ca^{2+}$  chelator, depends on influx of  $Ca^{2+}$  from extracellular medium. Selective muscarinic antagonists, pirenzepine (M1) and methoctramine (M2), were used in order to determine which type of receptor plays a role in mediating the  $[Ca^{2+}]_i$  response. At equimolar concentrations pirenzepine inhibits the carbachol stimulated increase in  $[Ca^{2+}]_i$ , while methoctramine has little or no effect. In order to further characterize the carbachol-stimulated biochemical responses the formation of inositol phosphates (IP's) were measured. Carbachol causes a two-fold increase of IP's over atropine pretreated or control cultures. These classical studies have indicated the presence of a muscarinic receptor that is similar in function to the M1 or M3 receptor, although the presence of a second type cannot be excluded. Future studies will examine the effect of carbachol on cAMP formation, expression of oligodendrocyte-specific markers (CNP, MBP, MAG), their phosphorylation states, and identification of the mAChR subtypes.



## SESSION 10: SECOND MESSENGERS

### POSTER ABSTRACTS

- P67 SUBTYPES OF MUSCARINIC RECEPTORS AND SIGNAL TRANSDUCTION IN CULTURED CELL LINES. B.V. Rama Sastry and C.C. Stephan. Departments of Anesthesiology and Pharmacology, Vanderbilt Medical Center, Nashville, TN 37232-2125

There are at least four subtypes (M1, M2, M3, and M4) of muscarinic receptors. Two signal transduction mechanisms, attenuation of hormone-stimulated cAMP formation and phosphatidylinositol (PI) hydrolysis, are coupled to M receptors. These two mechanisms are strongly expressed in two cell lines: (a) inhibition of cAMP formation in NG-108-15 neuroblastoma x glioma (NC) cells and (b) PI hydrolysis in 131-1N1 human astrocytoma (AC) cells. NG cells were prelabeled with [2-<sup>3</sup>H]-adenine in culture. cAMP formation was stimulated by PGE<sub>1</sub> (1 μM) and attenuated by carbachol (100 μM). The [<sup>3</sup>H]-cAMP formed was separated and analyzed. AC cells were prelabeled with [<sup>3</sup>H]inositol in culture. cAMP formation was stimulated by PGE<sub>1</sub> (1 μM) and attenuated by carbachol (100 μM). The [<sup>3</sup>H]-cAMP formed was separated and analyzed. AC cells were prelabeled with [<sup>3</sup>H]-inositol in culture. [<sup>3</sup>H]-Inositol-1-phosphate (IP) formed in the presence of LiCl (10 mM) and carbachol (100 μM) was separated and measured as an index of PI hydrolysis. The affinities (pA<sub>2</sub>) values for relatively selective muscarinic antagonists (Table 1) to block these effects of carbachol were determined. These observations indicate that M2 receptors strongly mediate cAMP attenuation while M3 receptors mediate PI hydrolysis. (Supported by The Council for Tobacco Research, US HHS NIDA DA 06207, and The Study Center for Anesthesia Toxicology.)

Table 1: pA<sub>2</sub> Values (affinities) of Muscarinic Antagonists

Agent	Relative M selectivity	cAMP (pA <sub>2</sub> )	PI hydrolysis (pA <sub>2</sub> )
Atropine	Non-selective	9.0	8.8
Pirenzapine	M1 selective	6.2	6.8
Methoctramine	M2 selective	7.6	6.0
HHSiD	M3 selective	6.9	7.6

- P68 ACETYLCHOLINE INDUCTION OF *zif/268* IMMEDIATE-EARLY GENE IS TRANSDUCED BY PLATELET-ACTIVATING FACTOR IN A NEURAL CELL LINE. Nicolas G. Bazan and John P. Doucet, LSU Eye Center and Neuroscience Center, 2020 Gravier Street, Suite B, New Orleans, Louisiana, USA.

Platelet-activating factor (PAF) is a membrane-derived second messenger synthesized in response to various stimuli. In neural tissue, PAF has been implicated in ischemia-reperfusion damage and is synthesized in brain following pharmacologically or electrically-induced seizures. PAF is synthesized in retina *ex vivo* and in fetal brain cells *in vitro* following stimulation with acetylcholine. High-affinity microsomal PAF binding sites in brain suggest that certain activities of PAF may be intracellular. *zif/268* (TIS8, *egr-1*, *krox24*), is an immediate-early gene encoding a DNA-binding, zinc finger transcription factor that has been linked to the establishment of long-term synaptic potentiation. In neurohybrid NG108-15 cells, acetylcholine stimulates induction of *zif/268* through an atropine-sensitive mechanism. The hetrazepine PAF antagonist BN50730, which is selective in displacing PAF from intracellular binding sites in brain, effectively antagonizes the induction of *zif/268* expression stimulated by acetylcholine. Results suggest that the genomic effects of acetylcholine stimulation in neural cells may be transduced through PAF within the neural cell. Therefore, lipid second messengers may link cholinergic neurotransmission to plasticity responses.

## SESSION 11: MODULATION OF INFORMATION

### SYMPOSIUM ABSTRACTS

S42 CHOLINERGIC MODULATION OF SENSORY INFORMATION. D.D. Rasmusson Dept. of Physiology & Biophysics, Dalhousie University, Halifax, N.S. Canada B3H 4H7

Recent studies have suggested that acetylcholine plays a role in enabling or permitting plasticity in other, non-cholinergic pathways. Sensory inputs to the visual, auditory or somatosensory cortex can be enhanced by direct application of acetylcholine onto cortical neurons or by activation of the cholinergic neurons innervating the cortex. Some of these changes outlast the treatment and are therefore possibly involved in memory storage. Suggestive evidence that acetylcholine is also necessary for some types of cortical plasticity comes from experiments in which cholinergic function has been blocked pharmacologically or the cholinergic basal complex has been lesioned. The relevance of this cholinergic modulation to the whole animal is speculative, but recordings from single cells in behaving animals or measurements of the release of acetylcholine from the cortex provide some directions to future research. Of particular interest are the conditions under which cholinergic neurons are activated and the input pathways by which this activation might take place.

S43 CHOLINERGIC AND GLUTAMATERGIC EFFECTS ON NEOCORTICAL NEURONS MAY SUPPORT RATE AS WELL AS DEVELOPMENT OF CONDITIONING. C.D. Woody and E. Gruen. UCLA Med. Ctr., MRR, BRI, Los Angeles, CA 90024.

Early studies<sup>1</sup> demonstrated that acetylcholine (ACh) could produce increases in excitability and input resistance of layer V neurons of the cat motor cortex that resembled changes found after Pavlovian eyeblink conditioning to paired click CS and glabella tap US<sup>2</sup>. Depolarization-induced discharge during ACh application made the changes long lasting, as were the changes after conditioning<sup>1,3</sup>. Intracellularly applied cGMP and cGMP-dependent protein kinase (cGPK) produced similar effects<sup>1,4</sup>. ACh and cGPK produce a decrease in a rapidly activated outward membrane current<sup>5</sup>, whose pharmacologic and voltage dependent properties resemble those of one of the family of A-currents<sup>6</sup>. The rate of Pavlovian conditioning can be increased by adding hypothalamic electrical stimulation (HS) to the CS and US<sup>7</sup>. HS produces a rapid cortical activation that is preferentially reduced by glutamate diethyl ester versus atropine. Locally iontophored glutamate (GLUT) can be paired with the same CS and US to produce rapid local conditioning of single unit spike activity. Both GLUT and HS must follow rather than precede CS-US delivery for conditioning to be successful. A decrease in a rapidly activated current similar to that reduced by ACh is found in units that have undergone local conditioning<sup>8</sup>. We conclude that interactions between ACh and GLUT may support changes in the rate of conditioned learning as well as the development of conditioning mediated by the decrease of an A-current.

1. Brain Res. 158:373, 1978.
2. J. Neurophysiol. 36:1104, 1973.
3. J. Neurophysiol. 44:605, 1980.
4. Brain Res. 386:379, 1986.

5. Brain Res. 424:193, 1987.
6. Brain Res. 480:72, 1989.
7. J. Neurophysiol. 49:767, 1983.
8. Brain Res. 539:76, 1991.

**SESSION 11: MODULATION OF INFORMATION**

**SYMPOSIUM ABSTRACTS**

- S44 THE CHOLINERGIC NEUROMODULATORY SYSTEM; AN EVALUATION OF ITS FUNCTIONAL ROLES. A.M. Sillito Dept of Visual Science, Institute of Ophthalmology, 11-43 Bath Street, London EC1V 9EL, UK**

The cholinergic system exerts a potent influence over the transfer of sensory information at thalamic and cortical levels. We have an understanding of the overall patterns of the cholinergic innervation at both levels in the system and good evidence concerning the way the main categories of inhibitory and excitatory cells in thalamic and cortical circuits may be influenced by the cholinergic terminals. It is possible to identify influences which seem to be most appropriately labelled as "modulating" the short term dynamics of the processing of sensory information in a way that links to shifts in the behavioural state along the axis from sleep to arousal. Equally there is evidence for longer term influences on synaptic transmission that may be necessary for plasticity in early life and conditioned response changes in the adult nervous system. To what extent can these latter influences be regarded as simply reflecting a requirement for the modulation of fast sensory processing to enable other influences to consolidate the changes? Can we distinguish between pattern and range of effects at thalamic and cortical levels in a meaningful way? Are there grounds for identifying distinctions in the pattern of effect between sensory and non-sensory systems?

## SESSION 11: MODULATION OF INFORMATION

### POSTER ABSTRACTS

- P69 TIME COURSE OF CHANGES IN RAT CORTICAL CHOLINERGIC INNERVATION AFTER UNILATERAL LESION OF THE NUCLEUS BASALIS WITH  $\alpha$ -AMINO-3-OH-4-ISOXOZOLE PROPIONIC ACID (AMPA). Marianita Calaminici, Fuad A. Abdulla, Jeffrey A. Gray, John D. Sinden & John D. Stephenson, Departments of Neuroscience and Psychology, Institute of Psychiatry, De Crespigny Park, London SE5 8AF.

A unilateral lesion of the nucleus basalis was made by injecting S-AMPA (10 mM) into the rostral and caudal parts of the nucleus (0.5  $\mu$ l at each site). The rats were killed by anaesthetic overdose at 2-3, 4-6, 8-10 weeks and 6 months after lesion and the brains fixed by transcardiac infusion of 4% paraformaldehyde and 15% saturated picric acid in phosphate buffer (0.1M, pH 7.4). Adjacent vibratome sections (30  $\mu$ M) were taken for cresyl fast violet, choline-acetyltransferase (ChAT) and acetyl-cholinesterase (AChE) staining. ChAT activity was demonstrated with anti-ChAT polyclonal antibody using an avidine-biotin/HRP protocol and AChE activity with acetylthiocholine iodide as the substrate and iso-OMPA as an inhibitor of non-specific esterase and diaminobenzidine intensification. The total length of AChE-staining axons per unit area were determined using an IBAS 2000 image analyzer. Briefly, the AChE-containing axons and cell bodies were discriminated from background, converted to a binary image and the cell bodies and any artefacts were eliminated by an interactive process. The axon images were thinned to 1 pixel and their total length expressed as a proportion of the field area. Two to 3 weeks after lesion there was a 90-95 % reduction in total axonal length in the frontal motor and somatosensory cortices of the lesioned hemisphere from approximately 110 mm mm<sup>-2</sup> to less than 11 mm mm<sup>-2</sup> with no significant recovery during the 6 month period after lesion. The loss of axons was not uniform throughout the ipsilateral cortex and in some cortical regions which did not receive their cholinergic innervation from the nucleus basalis (e.g cingulate cortex) there was a more than 60% increase in axonal density. Similar increases in axonal density were seen in cortical regions of the intact contralateral hemisphere. The findings suggest that the increases in ChAT activity which have been reported previously to occur in the contralateral nucleus basalis and cortex after unilateral neurotoxic lesions of the nucleus basalis and which were also seen in this study, are probably due to distant axonal sprouting.

- P70 TIME COURSE OF CHANGES IN THE SENSITIVITY OF RAT CORTICAL NEURONES TO MUSCARINIC RECEPTOR STIMULATION WITH ACh AND CARBACHOL AFTER UNILATERAL LESION OF THE NUCLEUS BASALIS WITH  $\alpha$ -AMINO-3-OH-4-ISOXOZOLE PROPIONIC ACID (AMPA). Fuad A. Abdulla, Marianita Calaminici, Jeffrey A. Gray, John D. Sinden & John D. Stephenson, Departments of Psychology and Neuroscience, Institute of Psychiatry, De Crespigny Park, London SE5 8AF.

Effects of unilateral lesions of the nucleus basalis with S-AMPA (10 mM, 2 injections of 0.5 $\mu$ l) on the sensitivities of frontal cortical pyramidal cells to iontophORIZED ACh, carbachol and glutamate were studied in anaesthetized rats 2, 3, 4, 6, 8-10 weeks and 6 months after lesion. Each drug was applied with an ejection current of 30 nA for 20 s and the averages of 3 applications (separated by 1 min intervals) were obtained. Control firing rates monitored over twenty 1 s epochs were compared with the firing rates during drug administration and for 20 s after ceasing drug application by a Wilcoxon signed ranks test. Neurones were considered to be sensitive when their firing rates increased or decreased ( $P < 0.05$ ) either during, or within 20 s of drug application. Only responses which were blocked by iontophoretic application of atropine were analysed. The percentage of neurones responding to ACh in the frontal cortex increased significantly 2 weeks after the lesion from 50% in the control rats to 86.7% in the lesioned rats ( $\chi^2 = 32.36$ ,  $P < 0.0001$ ) and this increase was still present at 6 months. The sensitivity of the frontal cortex neurones to carbachol was also significantly increased 2 weeks after the lesion, from 55.4% in the control group to 83.9% in the lesioned group ( $\chi^2 = 12.1$ ,  $P < 0.0005$ ). However, the sensitivity of the frontal cortex neurones to carbachol then declined gradually to 76.7% ( $\chi^2 = 7.04$ ,  $P < 0.005$ ) 3 weeks after the lesion, to 64.8% ( $\chi^2 = 1.25$ , n.s.) 4 weeks after the lesion and to 54.3% 6 weeks after the lesion and it stayed at that level for up to 6 months after the lesion. The sensitivity to glutamate did not change at any time. The differences in the time courses of sensitivity changes to ACh and to carbachol observed in neurones of the frontal cortex after lesioning the nucleus basalis could be explained by an enhanced response to muscarinic receptor stimulation which persisted for up to 3 weeks and a sustained decrease in acetylcholinesterase activity (see abstract by Calaminici et al., this meeting) which was responsible for the selective increase in response to ACh seen after 3 weeks.

## SESSION 11: MODULATION OF INFORMATION

### POSTER ABSTRACTS

- P71 **SENSORIMOTOR NEGLECT AND IMPAIRED SPATIAL PERFORMANCE IN RATS AFTER UNILATERAL LESION OF THE NUCLEUS BASALIS WITH  $\alpha$ -AMINO-3-OH-4-ISOXOZOLE PROPIONIC ACID (AMPA).** Fuad A. Abdulla, Mariarita Calaminici, Jeffrey A. Gray, John D. Sinden & John D. Stephenson, Departments of Psychology and Neuroscience, Institute of Psychiatry, De Crespigny Park, London SE5 8AF.

Impairment of sensorimotor function and of spatial learning were studied in rats 1, 8, 14 weeks and 6 months after unilateral lesion of the nucleus basalis with S-AMPA (10 mM, 2 injections of 0.5 $\mu$ l). The battery of tests used to assess sensorimotor function included measuring rotation in the open field, direction of rotation after tail pinch and placement on a 45° grid, and the responses to a light pin-prick, snout brushing, whisker touching and ammonia olfaction applied to one side of the body. Lesioned rats showed significant ipsilateral turning when placed in an open field, on a sloping grid and after tail pinch which persisted up to six months after the lesion. There was significant neglect in the responses to unilaterally applied stimuli but this decreased with time and at 6 months after the lesion responses to snout brushing, whisker touching and ammonia had returned to normal. The ipsilateral turning and pin prick response present 1 week after lesion were significantly reduced by arecoline (1.0 mg/kg, i.p.); responses to snout brushing, whisker touching or ammonia olfaction were not affected. Spatial learning ability was determined using a Morris water maze and was markedly impaired at 8-10 weeks after lesion. The time taken by the rats to find the escape platform when tested after 14 days of training (2 trials/day) increased from approximately 6 s in control rats to 49 s in lesioned rats. These results contrast with those of Page et al. (1991) showing that bilateral lesions of the nucleus basalis produced by injections of smaller amounts of AMPA did not affect acquisition of this spatial task. The reason for this discrepancy is not known but may be due to the longer time interval in this study between lesion and test (8-10 weeks compared with one week) or to differences between a large unilateral lesion and smaller bilateral lesions. The results confirm that the basal forebrain cholinergic system plays a role in learning and a more general role in other cortical functions.

Page, K.J., Everitt, B.J., Robbins, T.W., Marston, H.M. and Wilkinson, L.S. (1991). *Neuroscience*, 43, 457-472.

P72

**ASSOCIATING CHOLINERGIC AND SCHAFER COLLATERAL STIMULATION INITIATES PROTEIN SYNTHESIS IN CA1 PYRAMIDAL CELL DENDRITES.** P. Lipton & S. Feig. Depts. Physiol. Anat. U. Wisconsin Med. Sch. Madison, WI 53706.

A large body of evidence suggests that protein synthesis in the hippocampus around the time of learning is essential for memory consolidation. One way in which such synthesis might be involved is if associated synaptic inputs activate protein synthesis in the ribosomes which are located in subsynaptic regions of dendrites. Such a process could have great specificity.

Schaffer collaterals in the guinea-pig hippocampal slice were stimulated intermittently at 10 Hz over 20'. The cholinergic agonist carbachol (50  $\mu$ M) was added to the bath to mimic the effect of cholinergic stimulation. Protein synthesis in pyramidal cell layer dendrites, and somata, was measured autoradiographically, following 3' exposure to (3H)-leucine (to prevent dendritic flow) at the end of the 20' period.

Cycloheximide-dependent (3H) incorporation (protein synthesis) was negligible in the resting state and was not activated by Schaffer collateral stimulation, or carbachol, when administered separately. However, associating the two stimuli increased incorporation in dendrites 3-fold, without increasing uptake of free (3H)-leucine. The increase was cycloheximide-dependent (protein synthesis). There was no effect of the associated stimuli on protein synthesis in the cell somata. The increased synthesis in dendrites was completely blocked by the muscarinic antagonist, atropine, and by the NMDA-antagonist, D-APV.

Thus, associating inputs which may occur together during learning initiates protein synthesis in target dendrites of CA1 pyramidal cells. This is dependent on activation of NMDA receptors.

SYMPOSIUM ABSTRACTSS45 DOES THE BASAL FOREBRAIN(BF) CHOLINE(ACh)RGIC SYSTEM  
REALLY PLAY A ROLE IN MEMORY?Toshitaka Nabeshima ; Nagoya Univ. Sch. Med., Nagoya, Japan

In the ventromedial corner of the rat globus pallidus there is a group of large, cholinesterase(AChE)-reactive neurons, referred to BF such as the nucleus basalis or nucleus basalis magnocellularis, and believed to be homologous to the nucleus basalis of Meynert in human. A considerable body of evidence indicates that these cells are a major source of the extrinsic AChrgic input to the neocortex. An AChE-reactive fiber pathway originating in this region and terminating in cerebral cortex. Senile dementia of the Alzheimer type, a disorder characterized by a progressive deterioration of cognitive functions, appears to be related to a degeneration of AChrgic neurons in the forebrain, from findings that a marked decrease in cholineacetyltransferase(CAT) activity is observed in the hippocampus and widespread areas of the cortex in affected patients, and that a profound loss of neurons exists in the BF. Pharmacological modulations of the brain's AChrgic systems alter memory function. Anticholinergics, like scopolamine and atropine, disrupt learning and memory possibly by blocking the M-1 muscarinic receptor subtype(AChR) and induce a transient amnesia. On the other hand, both oxotremorine, a AChR agonist, and physostigmine, an anti-AChE, facilitate learning and memory and reverse experimentally induced memory deficits. Pharmacological studies, however, cannot explain the neural mechanisms responsible for the observed behavioral deficits. The diffuseness of the projection from the BF, the relatively compact distribution of the cells of origin, and the apparent utilization of ACh as a neurotransmitter by these cells provide the opportunity to study the relationship between brain ACh and behavior with a new research strategy. That is, destruction of this neuronal population makes it possible to examine the role in behavior played by these AChrgic neurons and their cortical innervation. In this concept, a variety of behavioral tests and observations have been done in order to assess the animal's cognitive capabilities and measured biochemical parameters such as CAT and AChE after the BF lesion by using electricity and neurotoxins. There are plenty of evidence for the behavioral deficits can be traced to specific damage in the BF. However, the heterogeneity of the BF and variations in the size and location of the lesion can results in different conclusions. So, in this Forum, the speakers will discuss whether the BF AChrgic system really plays role in memory.

S46 BASAL FOREBRAIN ACH AND MEMORY: DISCREPANCIES AND A POSSIBLE RESOLUTION  
Stephen B. Dunnett, Department of Experimental Psychology, University of Cambridge, Cambridge, U.K.

Electrolytic, kainic or ibotenic acid lesions of the NBM in the basal forebrain disrupt the performance of rats on a variety of learning/memory tasks, which has been interpreted as reflecting a cholinergic involvement in memory. However, the failure to replicate these deficits with lesions made by other toxins (quisqualate, AMPA) even though the destruction of NBM cholinergic neurones is at least as great, throws the cholinergic hypothesis into question and suggests instead that the deficits are attributable to damage of other non-cholinergic cells of the basal forebrain (most likely involving descending cortico-striato-pallidal projections). Nevertheless, other data does suggest a cholinergic involvement in memory processes: the induction of delayed matching deficits by injection of muscarinic antagonists into the cortex or hippocampus, and the reversal of maze learning and delayed matching deficits by cholinergic grafts in old rats. These discrepancies can be reconciled in the following hypotheses:

1. The ascending cholinergic systems do not mediate memory processes *per se*; rather they regulate cortical (and hippocampal) targets subserving distinct aspects of memory. The pattern of deficit after basal forebrain lesion will therefore be dependent upon which areas of the cortex lose their cholinergic inputs.
2. Prefrontal, cingulate and entorhinal areas of neocortex and the hippocampus are involved in short-term memory function, which is disrupted by antagonism of cholinergic inputs to those areas, but they are spared by conventional NBM lesions.
3. NBM lesions in rats deafferent the dorsal and lateral frontoparietal cortex, which do not subserve primary mnemonic processes, and mnemonic consequences of lesions here are indeed due to non-specific damage.
4. Electrolytic, kainic or ibotenic acid lesions of the basal forebrain disrupt efferent projections of the prefrontal and cingulate cortices, thereby disrupting performance on memory-related tasks, even though the cholinergic neurones of the NBM in that particular area are not implicated.

**SESSION 12:****BEHAVIORAL ASPECTS OF CHOLINERGIC TRANSMISSION****Forum for discussion:****SYMPOSIUM ABSTRACTS****S47 CHOLINERGIC MECHANISMS IN LEARNING AND MEMORY: IMPLICATIONS FOR ALZHEIMER'S DISEASE****H.C. Fibiger**, Division of Neurological Sciences, University of British Columbia

Systemic administration of drugs that affect neurotransmission at muscarinic synapses in the central nervous system have been shown repeatedly to influence the acquisition and post-acquisition performance of a variety of learned tasks. These findings suggest that central cholinergic systems serve important functions in some aspects of learning and memory. Because nearly the entire neuraxis is innervated by cholinergic neurons it has been difficult to ascribe mnemonic functions to specific cholinergic systems. The discoveries in the early and mid 1980's that Alzheimer's disease is associated with neuropathology in the cholinergic basal nuclear complex (CBC), which includes the nucleus basalis (nBM), and that ibotenic acid lesions in the region of these neurons produces learning and memory deficits in rats focussed attention on the CBC as a likely candidate. However, subsequent behavioral studies with other excitotoxins that by neurochemical criteria more extensively damaged the cortical projections of the nBM have cast doubt on the validity of this hypothesis. At present, therefore, the role of the telencephalic projections of the CBC in learning and memory remains uncertain. In the absence of selective toxins for cholinergic neurons, this issue has been difficult to resolve because even excitotoxins such as AMPA that clearly show greater selectivity for nBM neurons than does ibotenic acid cannot be assumed to spare completely non-cholinergic neurons in the vicinity of the nBM. If future studies demonstrate that the CBC and its projections do indeed serve important mnemonic functions, the question remains as to whether cholinergic replacement strategies will be useful in the symptomatic treatment of Alzheimer's disease. In contrast to the unequivocal success of such a strategy in Parkinson's disease, its application to Alzheimer's disease is potentially limited by a number of factors, including evidence that the putative postsynaptic target structures (hippocampus and cortex) are themselves damaged in this condition. While it remains possible that some therapeutic benefit will be generated by increasing cholinergic activity in these cytoarchitecturally disorganized structures, on the basis of current knowledge this seems unlikely. Appropriate clinical trials with procholinergic drugs in patients with Alzheimer's disease are required to resolve this issue.

**POSTER ABSTRACTS**

- P73 **Ch5 CHOLINERGIC NEURONS AND HYPOTHALAMIC REWARDING BRAIN STIMULATION IN RATS.**  
J.S. Yeomans, A. Mathur and M. Tampakeras, Department of Psychology,  
University of Toronto, Toronto, Canada M5S 1A1.

Ch5 cells of the pedunculopontine tegmental nucleus (PPN) are believed to monosynaptically activate dopamine cells of the ventral tegmentum (Bolam et al., 1991; Lacey et al., 1991). Cholinergic agonists injected near A10 dopamine cells improve conditioned place preferences or rewarding brain stimulation (Yeomans et al., 1985; Redgrave & Horrell, 1976), while muscarinic antagonists block rewarding brain stimulation (Yeomans et al., 1985; Kofman & Yeomans, 1988; Kofman et al., 1990). Lesions of medial PPN block opiate reward in a conditioned place preference task (Bechara & Van der Kooy, 1989) and inhibit rewarding brain stimulation (Buscher et al., 1989). Because Ch5 and Ch6 cells are inhibited by muscarinic agonists (Leonard & Llinas, 1988), we injected carbachol (1-4 ug) near Ch5 cells unilaterally in rats bar pressing for lateral hypothalamic stimulation. Carbachol raised thresholds by 400 to 1000% for 5-20 min on both sides of the brain followed by gradual recovery, without reducing bar-pressing rates in above-threshold tests. The most effective sites were in medial PPN. Scopolamine (100 ug) in these sites improved reward, i.e. decreased thresholds by 30 to 80%, for several hours to several days. This facilitation of rewarding brain stimulation by PPN scopolamine is greater than by peripheral amphetamine, and is accompanied by behavioral activation. The facilitation was blocked by atropine (60 ug) injected near A10 DA cells. We propose that hypothalamic rewarding brain stimulation is mediated by excitation of medial Ch5 cells, which then monosynaptically excite A10 dopamine cells.

(Supported by NSERCC grant A7077 to J.S.Y)

- P74 **CHANGES IN ACETYLCHOLINESTERASE ACTIVITY AFTER ADMINISTRATION OF CONVULSANT 3-MERCAPTOPROPIONIC ACID.**

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Instituto de Biología Celular, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155 - 1121 Buenos Aires, Argentina.

Cholinesterase inhibitors are known to lower thresholds for seizures induced by convulsant drugs. Here, the effect of the convulsant 3-mercaptopropionic acid (MP, 150 mg/kg, i.p) on acetylcholinesterase (AChE) activity in several areas of rats CNS was studied. As controls, rats injected with saline were used. Enzyme activity was assayed in membrane fractions from cerebral cortex, striatum, hippocampus and cerebellum, in absence and presence of ethopropazine (EP), a butyrylcholinesterase inhibitor. EP reduced AChE activity (20 - 30 %) in all the fractions studied. In cerebral cortex MP administration produced an increase (30 %) in total AChE activity but no changes were found if the enzyme was assayed in the presence of EP. In hippocampus an increase (30 %) in AChE activity was observed both in the absence and presence of EP. In cerebellum and striatum AChE activity decreased (20-30 %) when it was assayed in the absence and presence of EP. AChE activity in striatum membranes after saline injection was 50 % higher than in uninjected animals, regardless of EP presence during the assay. These results suggest that AChE activity changes are related to the participation of the studied CNS areas in generation and propagation of seizures.



## POSTER ABSTRACTS

## P75 INVOLVEMENT OF THE CHOLINERGIC SYSTEM IN ANTIAMNESIC EFFECTS OF NEFIRACETAM (DM-9384)

Toshitaka Nabeshima<sup>1</sup>, Masayuki Hiramatsu<sup>2</sup>, Tsutomu Kameyama<sup>2</sup>, Tadashi Shiotani<sup>3</sup>; <sup>1</sup>Nagoya Univ. Sch. of Med., Nagoya, <sup>2</sup>Meijo Univ. Fac. Pharmac. Sci., Nagoya & <sup>3</sup>Daiichi Pharmac. Co., Ltd., Tokyo Res. & Develop. Ctr., Tokyo, Japan.

Effects of nefiracetam (DM-9384, Daiichi Pharmac. Co., Ltd.) on learning and memory, choline acetyltransferase (CAT) activity, acetylcholine (ACh) release, [<sup>3</sup>H] QNB binding and choline uptake in normal and/or experimental amnesia animals were investigated. The step-down latencies (SDL) in passive avoidance of the CXM (30 and 60mg/kg, s.c.) groups were significantly lower than those of the saline-treated control group 24h after training. DM-9384 alone failed to change SDL of naive mice. However, DM-9384 showed anti-amnesic effects on the CXM-induced amnesia. The effects of DM-9384 was antagonized by scopolamine (1mg/kg, i.p.). CXM decreased Bmax of [<sup>3</sup>H] QNB binding in the whole brain excepting cerebellum and CAT activity in the cerebral cortex 12.4 and 4.3%, respectively 24h after treatment. These decreases were protected completely by DM-9384. Memory deficiency occurred when mice were exposed to CO immediately after training (acute amnesia), at 7 days before training and 7 days after training (delayed amnesia). DM-9384 (5 and 10mg/kg) prolonged the SDL in the CO-induced amnesia group. Scopolamine blocked the anti-amnesic effects of DM-9384 on delayed amnesia. Electrolytic lesion of the basal forebrain (BF) of rats produced a learning deficit in the multiple T-maze and passive avoidance tasks. DM-9384 (1 and 3mg/kg) attenuated the BF-lesion-induced amnesia in both tasks. DM-9384 (3mg/kg) showed a tendency to counteract the decrease of CAT activity in the fronto-parietal cortex induced by BF-lesion. In the naive rats, 14 days repeated administration of DM-9384 (1,3 and 10mg/kg) increased CAT activity in the cortex about 10%. Acute administration of DM-9384 (1,3 and 10mg/kg) increased ACh release in *in vivo* dialysis study. [<sup>3</sup>H] choline uptake was also increased by DM-9384. The dose response curves of DM-9384 were bell-shaped in both behavioral and biochemical studies. These results suggest that DM-9384 potentiates the AChergic neuronal function and produces its anti-amnesic effects.

## P76 AGE-DEPENDENT EFFECT OF AF64A ON CHOLINERGIC PARAMETERS IN THE RAT BRAIN: ARE AGED ANIMALS LESS SENSITIVE?

El Tamer, A., Wulfert, E. and Hanin I.; Loyola Univ. Chicago Stritch Sch. Med., Dept. Pharmacol., Maywood, IL 60153 and \*UCB s.a. Pharmaceutical R&D, Brussels, Belgium.

Our previous studies have demonstrated that intracerebroventricular (icv) administration of low doses of ethylcholine mustard aziridinium (AF64A), up to 1.0 nmol/side, induces a reversible cholinergic deficit in the hippocampus, paralleled by a compensatory increase in choline acetyltransferase (ChAT) activity in the septum (El Tamer et al. Neuropharmacol. 1992, 31, 397-402). In the present study we have addressed the question as to whether this effect might change with the age of the animal. Three groups of rats aged 4, 12 and 22 months, respectively (4 rats per group) were injected icv with AF64A (0.5 nmol/side). Seven (7) and 14 days post-injection, animals were decapitated and septum and hippocampus were dissected out of the brain. ChAT and acetylcholinesterase (AChE) activities were determined according to procedures described in the previous study. In the hippocampus ChAT activity was decreased to the same extent (-23%;  $P < 0.01$ ) in the three groups of rats, whereas AChE was decreased to a similar extent (-26%;  $P < 0.01$ ) in the 4 and 12 month old groups but remained unchanged in the 22 month old group. In the septum, there was no effect on AChE activity in the 3 age groups, whereas ChAT was increased significantly (+18%;  $P < 0.05$ ) in the 4 and 12 month old groups only. By 14 days post-injection of AF64A, ChAT and AChE activities were back to normal in all three groups and in both brain areas. We conclude that the difference in AF64A's effect between the 22 month old group and the two other groups might reflect an age-dependent change in the sensitivity of the cholinergic system toward AF64A.

POSTER ABSTRACTS**P77 REGIONAL DIFFERENCES IN [ $^3$ H]-QUINUCLIDINYL BENZILATE BINDING TO CNS MEMBRANES AFTER 3-MERCAPTOPROPIONIC ACID INDUCED-SEIZURES.**

Patricia G. Schneider and Georgina Rodríguez de Lores Arnaiz. Instituto de Biología Celular. Facultad de Medicina. U.B.A. 1121-Buenos Aires. Argentina.

We have already demonstrated that the administration of the convulsant 3-mercaptopropionic acid (MP) to rats (150 mg/kg, i.p.) increases the muscarinic receptor affinity to [ $^3$ H]-quinuclidinyl benzilate ([ $^3$ H]-QNB). The increase was observed in cerebellum and striatum membranes, both in seizure and postseizure states. No modifications in cerebral cortex and hippocampus were found. To study the time course and the possible reversibility of MP effect, we employed a lower dose (50 mg/kg), which also produces seizures. The stages studied were: pre seizure, seizure, early and late postseizure (at 4 min, 7-11 min, 25-30 min and 24 h after injection, respectively). [ $^3$ H]-QNB binding to cerebellum membranes increased at pre seizure (+27%), seizure (+58%) and early postseizure (+20%), but decreased at late postseizure (-20%). Though smaller, increases proved reversible in cerebral cortex and hippocampus. MP "in vitro" ( $10^{-4}$  M) failed to modify [ $^3$ H]-QNB binding to cerebellar membranes. Results indicate that the increase in [ $^3$ H]-QNB binding by MP is reversible in time and selective for certain CNS areas, but seems independent of drug presence alone. These findings support the participation of cholinergic CNS pathways in the development and maintenance of MP seizures.

**P78 INTERACTIONS BETWEEN GLUCOSE, ACETYLCHOLINE SYNTHESIS AND MEMORY PROCESSES.** C. Messier, T. Durkin, P. de Boer\*, B.H.C. Westerink\* and C. Destrade\*. School of Psychology, U. of Ottawa (Ont.), CANADA, \*Lab. Neurosci. Cognitives et Comport., URA CNRS 339, U. de Bordeaux I, FRANCE and +Univ. Ctr. for Pharmacy, U. of Groningen, HOLLAND.

Peripheral and central glucose injections have been shown to improve memory both in animals and humans and to attenuate scopolamine-induced amnesia in animals. Because acetylcholine (ACh) synthesis can, in some circumstances, depend on the availability of glucose from which is derived acetyl coenzyme A, one of the precursor of ACh, and because cholinergic function in the hippocampus is important for memory processes, we have studied the interaction between glucose and cholinergic function in the hippocampus. Peripheral injections of glucose (3g/Kg) attenuated the activation of sodium-dependent high-affinity choline uptake (HACU) induced either by learning or by a scopolamine injection (1 mg/kg, i.p.) while no effect on HACU levels was found in untrained or untreated mice. These results provided an indirect demonstration that when cholinergic neurons are activated, glucose can facilitate ACh synthesis. Using an in vivo intracerebral microdialysis technique, it was shown that a peripheral scopolamine injection (1 mg/kg) increased the ACh outflow from the hippocampus of rats (10-20 fold) and that a combined peripheral glucose injection (3g/kg) further increased scopolamine-induced ACh outflow. This result is a more direct demonstration that peripheral glucose injections can facilitate ACh synthesis and release during increased neuronal activity. Taken together, these results suggested that the memory-improving action of glucose may depend on this facilitation of ACh synthesis. To further test this hypothesis, we examined the effect of insulin injections on scopolamine-induced amnesia. We speculated that, if glucose protected from scopolamine amnesia, insulin should potentiate scopolamine amnesia. Contrary to our predictions, the results showed that a post-training 0.8 I.U./Kg insulin injection attenuated scopolamine-induced amnesia in mice while insulin injections alone had no effect on memory.

**SESSION 12:****BEHAVIORAL ASPECTS OF CHOLINERGIC TRANSMISSION**

Forum for discussion:

**POSTER ABSTRACTS****P79 CHOLINERGIC SPECIFICITY OF LESION-INDUCED DEFICITS AND DRUG-INDUCED IMPROVEMENTS IN RADIAL ARM MAZE MNEMONIC PERFORMANCE IN RATS.**

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In the first of two experiments Ibotenic (0.06M) and Quisqualic acid (0.12 or 0.18M) lesions to the Nucleus Basalis Magnocellularis (NBM) and the Medial Septal and Diagonal Band areas (MSA), produced comparable and highly significant long lasting impairments in rats performance of an 8-arm radial maze task. Choline Acetyltransferase (ChAT) activity was reduced by both agents to, on average, 60% of control level in frontal cortex and to 70% in hippocampus. Nicotine (0.05 and 0.10 mg/kg) and the beta-carboline antagonist / partial inverse agonist ZK93426 (1.25, 5.00, 10.00 mg/kg) produced similar dose-dependent decreases in errors in all lesion groups. Nicotine did not affect control errors and ZK93426 dose-dependently increased them. These results suggest that cholinergic lesions with different excitotoxins, at concentrations which lead to equivalent levels of ChAT depletion can produce analogous performance deficits. In the second experiment, animals with 6-hydroxydopamine lesions to the Dorsal Noradrenergic Bundle (DNAB) were seen to be impaired with respect to controls, in radial maze performance ; but errors, although high, were less consistent and, overall, lower than in an ibotenate lesioned group, despite levels of noradrenalin depletion of over 90% in frontal cortex and over 50% in hippocampus. Nicotine and ZK93426, at the above doses, again dose-dependently decreased errors in the ibotenate group but both had no effect on errors made by the DNAB group. In conclusion, it is suggested that increases in maze errors after lesions to the basal forebrain reflect cholinergic dysfunction, which can be reversed by either direct (nicotine) or indirect (ZK93426) enhancement of cholinergic transmission. However, impairment of radial maze performance induced by noradrenergic damage is insensitive to cholinergic manipulations.

**P80 PHOSPHATIDYLSERINE TREATMENT ATTENUATES THE CHOLINERGIC IMPAIRMENT INDUCED BY AGING AND NUCLEUS BASALIS MAGNOCELLULARIS LESIONS.**

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In vivo basal acetylcholine (ACh) release and choline (Ch) efflux in the cortex, hippocampus and striatum of 20-22-month-old rats, investigated by means of transversal dialysis, were 50-70 % and 20-30 % lower than in young rats, respectively. Also the increase in ACh release elicited by 100 mM KCl depolarization was significantly smaller than in young animals. These decreases were attenuated by daily administrations of 15 mg/Kg i.p. of either brain-bovine phosphatidylserine (BC-PS) or 1,2-diacyl-s-glycero-3-phospho-L-serine (PS-S1). BC-PS reversed basal and 100 mM KCl ACh release and Ch output in the cortex, hippocampus and striatum. PS-S1 reversed the age associated decrease in basal ACh in the hippocampus but not in the cortex and striatum, and restored Ch efflux and KCl-evoked ACh release in all regions investigated. In 3- and 18-month-old rats, 1 week after unilateral quisqualic acid lesion of the nucleus basalis magnocellularis (NBM), ACh release in vivo and cholinergic acetyltransferase (ChAT) activity in the ipsilateral cortex were 50% lower than in control rats. Preliminary results demonstrate that after 8 days of BC-PS treatment (15 mg/kg/die i.p.) both the decrease in ACh release and ChAT activity were attenuated in aged but not in young lesioned rats.

Supported by a grant from CNR target project on aging.

# SESSION 12:

# BEHAVIORAL ASPECTS OF CHOLINERGIC TRANSMISSION Forum for discussion:

## POSTER ABSTRACTS

- P81 **MUSCARINIC RECEPTORS IN THE CEREBRAL CORTEX OF NBM-LESIONED RATS WITH CHROMAFFIN CELL GRAFTS.** Welner S.A., Koty Z.C., Aubert, I. and Justino, H. Douglas Hospital Research Centre, Department of Psychiatry, McGill University, Montreal, Quebec, Canada H4H 1R3.

Lesions of the nucleus basalis magnocellularis (NBM) in rats produce spatial memory deficits as well as changes to cholinergic markers in the cerebral cortex, the target area of projections from the NBM. We have previously reported that adrenal chromaffin cells transplanted to the cerebral cortex of rats with NBM lesions are able to ameliorate lesion-induced spatial memory deficits and increase acetylcholinesterase staining in the host cortex (Welner et al., *Brain Research*, 527: 163, 1990). In the present experiments, we test whether grafting chromaffin cells to the cerebral cortex of NBM-lesioned rats will have an effect on muscarinic receptors in the cerebral cortex of NBM-lesioned rats. Male Sprague-Dawley rats were pre-trained in a T-maze to 75% correct responding; three quarters of the group were lesioned bilaterally in the NBM and, 2 weeks post-lesion, one quarter were grafted with suspensions of chromaffin cells (LCG) and one quarter with kidney cells (LKG), as a control. Cells for grafting were obtained from adult donor rats. Three months post-grafting, rats were retested in the T-maze for spatial memory behavior and their brains frozen for receptor analysis. M<sub>2</sub> binding sites were measured using [<sup>3</sup>H]AF-DX 384 and M<sub>1</sub> binding sites using [<sup>3</sup>H]pirenzepine by both membrane binding in whole cortex and autoradiography. Behaviorally, previous results were confirmed in that chromaffin cell grafts reversed lesion-induced spatial memory deficits; implantation of kidney cell grafts did not. In membrane binding studies, no difference was measured in either the K<sub>d</sub> or B<sub>max</sub> for any treatment group. Alternatively, when brains were analyzed by autoradiography of more discrete areas surrounding the graft sites, certain trends were observed. M<sub>2</sub> sites, which are mostly pre-synaptic, were slightly decreased in the lesion group and increased in the LCG group; LKG animals showed intermediate effects. For M<sub>1</sub> sites, which are mostly post-synaptic, an increase in sites was measured in the lesion group and a decrease in this level in LCG and LKG groups. These results would indicate that M<sub>2</sub> sites may be the more relevant for memory-related behaviors.

- P82 **INCREASE IN MUSCARINIC-M2 RECEPTOR BINDING SITES IN CERTAIN BRAIN REGIONS OF AGED MEMORY-IMPAIRED AS COMPARED TO MEMORY-UNIMPAIRED RATS.** I. Aubert, W. Rowe, M.J. Meaney, and R. Quirion. Douglas Hospital Research Center and Departments of Neurology & Neurosurgery and Psychiatry, McGill University, Montreal, Quebec, Canada, H4H 1R3.

Long-Evans rats (24 months) were classified into cognitively impaired (n=10) or unimpaired (n=10) subgroups based on their spatial learning ability in the Morris Swim Maze task. Using quantitative *in vitro* receptor autoradiography, the status of muscarinic M<sub>1</sub> ([<sup>3</sup>H]pirenzepine, 15nM) and M<sub>2</sub> ([<sup>3</sup>H]AF-DX 384, 2nM) receptor binding sites was investigated. No statistical differences were observed in the muscarinic M<sub>1</sub> receptor subtype in the aged impaired as compared to the aged unimpaired group. In contrast, a significant increase in putative muscarinic [<sup>3</sup>H]AF-DX 384/M<sub>2</sub> binding sites was observed in the frontoparietal cortex, the striatum and especially in the dentate gyrus of the hippocampus in the aged impaired group. No significant differences in the number of M<sub>2</sub> binding sites were observed in any layers of the CA1 region of the hippocampus and in the medial septum. Since muscarinic M<sub>2</sub> receptors are considered to act as negative autoreceptors, an augmentation of their densities could cause a decrease in acetylcholine release, this possibly leading to memory impairments as observed here in the aged impaired group.

Supported by the Alzheimer Society of Canada, Alcan Corporation and MRCC.

## SESSION 13: CHOLINERGIC INVOLVEMENT IN SLEEP AND AROUSAL

### SYMPOSIUM ABSTRACTS

- S48 CHOLINERGIC RECEPTOR SUBTYPES AND REM SLEEP IN ANIMALS, NORMAL CONTROLS, AND PSYCHIATRIC DISORDERS  
J. Christian Gillin, M.D., Rafael Salin-Pascual, Javier Velazquez-Moctezuma, Peter Shiromani, Ph.D., and Rebecca Zoltoski, Ph.D.  
University of California, San Diego and San Diego Veterans Affairs Medical Center

Appropriate systemic administration of nonselective cholinergic agonists, such as physostigmine, arecoline, RS 86, and pilocarpine, induce REM sleep in normal controls. The REM sleep enhancing effects of arecoline can be blocked by pretreatment with scopolamine, a nonselective muscarinic antagonist. Likewise, REM sleep is induced by administration of relatively nonselective cholinergic agonists, such as carbachol, neostigmine, or bethanachol, into medial pontine reticular formation (MPRF) in cats. The effect of carbachol is blocked by pretreatment with atropine. Repeated daily administration of scopolamine in man and rats shows that tolerance develops quickly to the REM sleep suppressing effects, while a REM sleep "rebound" occurs with abrupt discontinuation. Recently, with the intracerebral administration of relatively selective muscarinic  $M_2$  agonists (oxotremorine-M and cisdioxolene) and  $M_1$  antagonists (pirenzepine) in cats, we have suggested that drug-induced enhancement of REM sleep in mPRF is mediated by  $M_2$  receptors. Moreover, we have found that nicotine administration in mPRF also elicits REM sleep. Nevertheless, the role of  $M_1$  receptors can not be ruled out: systemic administration of biperiden, a relatively selective  $M_1$  antagonist, inhibits REM sleep in both humans and cats.

- S49 REM SLEEP INDUCTION BY CHOLINERGIC MICROSTIMULATION: LOCUS AND CHARACTERISTICS OF SHORT AND LONG TERM ENHANCEMENT SITES IN THE PONTINE TEGMENTUM OF THE CAT. J.A. Hobson, S. Datta, J. Calvo and J. Quattrochi. Department of Psychiatry, Harvard Medical School.

For many years it has been known that the microinjection of Carbachol into the paramedian pontine tegmentum promptly induces a REM sleep-like state. The effective zone for this short-latency effect extends from the mesencephalic border anteriorly to the pontomedullary junction posteriorly. An optimal site (defined as the shortest latency, largest percent increase, and qualitatively most REM-like syndrome) is in the anterodorsal pontine tegmentum at a point medioventral to the locus coeruleus and lateroventral to the dorsal raphe nucleus, a zone which is devoid of either aminergic or cholinergic neurons. Because the effects are immediate but short-lived, we call this region the short term REM sleep induction site (STR).

We have recently discovered that carbachol microinjection into more lateral and posterior sites in the peribrachial pons produces a REM sleep increase that is equally intense but is both delayed and prolonged. Because these effects peak on the second or third day post injection and only return to baseline 10 days later, we call this region the long-term REM sleep induction site (LTR). We had previously discovered - and have now reconfirmed - the fact that cholinergic stimulation of the LTR produces an immediate enhancement of stereotyped clusters of EEG waves, in the lateral geniculate body and induces firing of local bursting cells prior to each wave. These so-called "PGO" waves are normally restricted to REM sleep but now appear in all states in the thalamus ipsilateral to the stimulation site in the pons.

The differential response to carbachol at the two sites suggests: 1. that REM sleep may be mediated (or triggered) via cholinceptive activation of an executive pontinal neuronal population (that is very extensive and includes both the STR and LTR) and 2. that the regulation (or set point) of the systems may be mediated in part by the neurons of the LTR.

## SESSION 13: CHOLINERGIC INVOLVEMENT IN SLEEP AND AROUSAL

### SYMPOSIUM ABSTRACTS

- S50 CHOLINERGIC BLOCKAGE OF INTRINSICALLY- AND NETWORK-GENERATED SLOW OSCILLATIONS PROMOTES WAKING AND REM-SLEEP PATTERNS IN THALAMIC AND CORTICAL SYSTEMS. Mircea Steriade, Laboratoire de Neurophysiologie, Faculté de Médecine, Université Laval, Quebec, Canada G1K 7P4.

The thalamus and cerebral cortex generate three major rhythms of brain electrical activity during the state of sleep with EEG synchronization (S). 1. Spindles (7-14 Hz), the epitome of EEG synchronization at sleep onset, are produced in the network comprising reticular (RE) and thalamocortical cells. 2. Delta waves (1-4 Hz) occur during later stages of sleep through the interplay between two intrinsic electrophysiological properties of dorsal thalamic neurons. As delta oscillations appear at a membrane potential more negative than spindles, I postulate a progressive hyperpolarization of thalamocortical cells across S (due to the decreased firing rates of cholinergic and aminergic neurons), with the consequence of shifting spindling to delta activity. 3. Slow (0.1-0.5 Hz) oscillations appear as network-generated, rhythmic depolarizing envelopes in pyramidal cortical cells. I will propose that these slow cortical rhythms synchronously drive RE cells with the consequence of grouping both spindle and delta oscillations at a frequency of 0.1-0.5 Hz. Brainstem and basal forebrain cholinergic systems block spindles, delta as well as slower sleep rhythms and, thus, shift the activity of thalamocortical systems to waking and REM- sleep patterns, i.e. tonic single-spike firing, enhanced excitability, and (during waking) sculpturing inhibition with the preservation of short-lasting IPSPs involved in fine discrimination processes. The cholinergic suppression of spindling is due to the inhibition of the pacemaking RE neurons. Delta oscillations are suppressed by depolarization of thalamocortical cells, thus bringing them out of the voltage range where this rhythm is generated. The slow cortical rhythm is suppressed by the selective blockage of the hyperpolarization (probably due to a  $\text{Ca}^{2+}$ -dependent K current) separating the depolarizing envelopes. A unified view of these sleep rhythms and their cholinergic modulation during brain arousal will be presented.

- S51 A CRITICAL ROLE FOR CHOLINERGIC NEURONS IN WAKING AND PARADOXICAL SLEEP  
Barbara E. Jones, Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada.

Since the early pharmacological and biochemical studies of the late '50s and '60's, it has been known that acetylcholine (ACh) could play an important role in processes of central activation that occur during waking and paradoxical sleep. With the knowledge of the organization of central cholinergic systems, it has become possible to examine in detail the role of the brainstem and forebrain cholinergic neurons in these states. Within the brainstem, the cholinergic cells of the dorsolateral pontomesencephalic tegmentum provide an innervation to the brainstem reticular formation as well as give rise to important ascending projections, particularly to the thalamus. By action upon reticular and thalamic neurons, they appear to play a critical role in certain processes of activation, and particularly during paradoxical sleep, as the results of lesion studies would suggest. This role depends upon activation of large populations of non-cholinergic (probably glutamatergic) reticular neurons as well as interaction with monoaminergic neurons, possibly via GABAergic cells, within the brainstem. Within the forebrain, the cholinergic cells of the magnocellular basal nucleus provide an innervation to the cerebral cortex and receive in turn important afferent input from the brainstem reticular formation. Serving as the ventral extra-thalamic cortical relay of the ascending reticular activating system, these cholinergic neurons are differentially modulated by neurotransmitters originating from brainstem afferents. Together with GABAergic neurons with which they lie intermingled in the basal forebrain, they may mediate the changes in cortical activity that originate in the brainstem and are transmitted to the cortex over the sleep-waking cycle. By regular tonic activity, the cholinergic neurons may maintain cortical activation during the states of wakefulness and paradoxical sleep.

**SESSION 13: CHOLINERGIC INVOLVEMENT IN SLEEP AND AROUSAL**

**POSTER ABSTRACTS**

**P83 ULTRASTRUCTURAL EVIDENCE FOR SEROTONERGIC INPUT TO CHOLINERGIC NEURONS IN THE LATERODORSAL TEGMENTAL NUCLEUS IN THE RAT.**

Kazuo Semba and Takashi Honda

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We have recently shown that application of serotonin (5HT) decreases excitability and markedly modulates the discharge pattern of cholinergic bursting neurons in the laterodorsal tegmental nucleus (LDT) [Luebke et al., PNAS, 89(1992):743]. In the present study, we used electron microscopy to investigate synaptic relations between serotonergic axon terminals and cholinergic LDT neurons, by combining PAP-DAB pre-embedding immunohistochemistry for choline acetyltransferase (ChAT), and post-embedding immunogold staining for 5HT. ChAT-immunoreactive somata were mostly medium-sized and appeared oval, triangular or multipolar. Their somatic surface was covered by glial processes and both myelinated and unmyelinated axons. ChAT-positive neurons received many synaptic contacts on somata, dendrites and dendritic spines. In post-embedding immunogold-stained material, deposition of gold particles was seen in some of these axon terminals in contact with ChAT-immunoreactive neurons. These serotonergic synaptic contacts appeared to occur more frequently with somata and proximal dendrites than with distal dendrites or dendritic spines. The presence of serotonergic synaptic contacts predominantly with the somata and proximal dendrites of cholinergic LDT neurons is consistent with the suggested role of serotonin in modulating rapid eye movement sleep phenomenology through its action on cholinergic neurons in the mesopontine tegmentum.

**P84 MESOPONTINE CHOLINERGIC NEURONS AND FUNCTIONS OF MESOLIMBIC DOPAMINE NEURONS, INCLUDING SCHIZOPHRENIA. J.S. Yeomans, Department of Psychology, University of Toronto, Toronto, Canada M5S 1A1.**

Scopolamine (100ug) injections near medial Ch5 cells reduced thresholds for rewarding brain stimulation and increased behavioral activation, both functions consistent with overactivation of mesolimbic dopamine cells (see Yeomans, Mathur & Tampakeras abstract). Ch5 cells are believed to monosynaptically activate ventral tegmental dopamine cells via both muscarinic and nicotinic receptors (Bolam et al., 1991; Lacey et al., 1991; Clarke et al., 1987). Dopamine overactivation is also believed important in positive symptoms of schizophrenia (e.g. Seeman et al., 1976). It is proposed here that Ch5 overactivation can be a causal factor in some forms of schizophrenia. Ch5 and Ch6 cells were found in twice normal numbers in a small sample of schizophrenic brains (Karson et al., 1991). AChE activity is increased in many thalamic regions in some schizophrenics (McGeer & McGeer, 1977). Some schizophrenics have early onset REM sleep (Taylor et al., 1991), a sign of increased Ch5 cholinergic activity. It is proposed that increased numbers of Ch5 and Ch6 cells could be a predisposing hereditary factor that is exacerbated by increased cholinergic tone (as measured by delta-wave amplitude reduction) in early adulthood (Feinberg, 1982). Cholinergic overactivation, in turn, may result in disorganized thinking (via thalamic activation, e.g. Steriade, 1990), delusions and hallucinations (via mesolimbic dopamine activation) and REM sensitivity (via pontine reticular formation activation, e.g., George et al. 1964), all symptoms of schizophrenia.

(Supported by NSERCC grant A7077 to J.S.Y.)

## SESSION 14: CLINICAL ASPECTS OF CHOLINERGIC PHARMACOLOGY

### SYMPOSIUM ABSTRACTS

#### S52 CLINICAL ASPECTS OF CHOLINERGIC PHARMACOLOGY

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Just a few years ago the "cholinergic deficiency hypothesis of Alzheimer's disease" was the focal concept for many studies attempting to understand, and treat this devastating disease state. Recently there has been some erosion of the cholinergic concept, in view of clinical neurochemical evidence showing brain alterations in other neurotransmitters in Alzheimer's disease besides just acetylcholine - implying a more extensive, broader *in vivo* neuropharmacological dysfunction in this disease state. This, combined with a major recent research thrust toward a molecular initiative to elucidate the sources of origin of the characteristic plaques and tangles of Alzheimer's disease, have all served to diminish, in the eyes of at least some individuals, the importance of the role of cholinergic mechanisms in Alzheimer's disease. Nevertheless, memory deficits and dementia are both highly dependent on the presence of an intact cholinergic system in brain *in vivo*, and the treatment of these severe deficits in the Alzheimer patient is an essential component of the treatment strategy. The papers presented in this session will survey several different cholinergic approaches which have been attempted in the clinic. Their efficacy, practicality and utility will be addressed and critically evaluated.

#### S53 APPROACHES TO CHOLINERGIC THERAPY IN ALZHEIMER'S DISEASE

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Therapeutic approaches which aim to provide palliative symptomatic relief in Alzheimer's disease have focused on the "cholinergic hypothesis". Our own laboratory has developed a series of novel and highly potent muscarinic agonists which freely penetrate CNS (J. Saunders & S. Freedman, 1989, *TIPS* Supplement pp 70-75). The original compounds, however, were full agonists with no selectivity for muscarinic receptor subtypes. Their peripheral toxicity precluded their use clinically. More recently we have devised low efficacy compounds which display functional selectivity for muscarinic receptor subtypes. For example, L-689,660 (1-azabicyclo(2.2.2.)octane, 3-(6-chloropyrazinyl)maleate) is a potent full agonist at  $M_1$  receptors in superior cervical ganglia, a potent partial agonist at  $M_3$  receptors in ileum, but acts as a competitive antagonist at  $M_2$  receptors in atrial cardiac tissue. This compound consequently has reduced cardiovascular toxicity. A second compound, L-687,306 (3R,4R-3-(3-cyclopropyl-1,2,4-oxadiazol-5-yl)-1-azabicyclo[2.2.1]heptane) displays potent partial agonist properties at  $M_1$  receptors, but is an antagonist at both  $M_2$  and  $M_3$  receptors. Another approach to cholinergic therapy has been based on the clinical observation that the acetylcholinesterase inhibitors physostigmine and tacrine cause statistically significant improvements in cognitive function in patients with Alzheimer's dementia. A number of improved acetylcholinesterase inhibitors are in development which may prove to have clinical potential in Alzheimer's therapy.



## SESSION 14: CLINICAL ASPECTS OF CHOLINERGIC PHARMACOLOGY

### SYMPOSIUM ABSTRACTS

- S54 WHAT HAVE WE LEARNED FROM THE THA TRIALS TO FACILITATE TESTING OF NEW AChE INHIBITORS? S. Gauthier and L. Gauthier, McGill Centre for Studies in Aging and School of Physical & Occupational Therapy, McGill University.

As a long-acting AChE inhibitor, THA has been extensively studied since Summers report in 1981. Four published studies (Chatellier, 1990; Gauthier, 1990; Egger, 1991; Molloy, 1991) used cross-over designs, THA doses of 50 to 150mg/day with lecithin for 3 to 13 weeks. Although some statistically significant changes were found on cognitive scales such as Folstein's MMSE, none of the studies showed improvement in functional autonomy. Carry-over effects were detected even after 4 weeks of wash-out, whereas rebound deterioration occurred off THA. Peripheral side-effects reflected GI tract, bladder, sweat and salivary glands (but not cardio-vascular) cholinergic stimulation. Liver toxicity occurred in 13 to 41% of subjects. We learned that (1) parallel designs are preferable to cross-over, with different doses per group; (2) efficacy should be determined on first exposure to the drug, and must include a clinical global impression of change as well as a global cognitive scale; (3) new instruments sensitive to behavioral spontaneity must be developed.

- S55 BRAIN SELECTIVE INHIBITION OF CHOLINESTERASE: A NEW APPROACH TO THE THERAPY FOR ALZHEIMER'S DISEASE.

Albert Enz, Preclinical Research SANDOZ PHARMA Ltd. CH-4002 Basle/Switzerland.

The main neuropathological findings in Alzheimer's disease (AD) are the deposits of neuritic plaques and neurofibrillary tangles in postmortem brains. The acetylcholine (ACh) degrading enzyme acetylcholinesterase (AChE) is accumulated in these structures. Further, amongst other neurotransmitter systems, a marked decrease of cholinergic transmission due to a loss of cholinergic terminals in cortex and hippocampus characterizes this disease. Hence, the first attempt to treat the symptoms of AD substitutively was made with the ACh precursor choline almost twenty years ago. This approach as well as clinical trials using direct acting muscarinic agonists failed for several possible reasons, one of them being the prominent peripheral adverse cholinergic effects. The discovery and cloning of the five muscarinic receptors raised hope of finding selective agonists which should be active at the putative m1 site in AD brains. However until now, unfortunately, all efforts made towards the development of m1 selective agonists resulted in agonists with only low efficacy. Regarding the cholinergic support in AD brains the administration of AChE inhibitors (AChE-I) seemed to show marginal effects until now. Tacrine and physostigmine were investigated with some positive responses in AD patients. The lack of good efficacy with physostigmine was explained by the drug's short half life and its unpredictable bioavailability after oral administration, while the use of tacrine is limited by the occurrence of liver toxicity. In order to confirm the cholinergic hypothesis AChE-I's showing relatively high brain selectivity shared with low peripheral influence and toxicity therefore is needed. SDZ ENA 713 a novel long acting AChE-I, is a candidate which might fulfil such criteria. The action of brain selective AChE-I's can be seen with regard to a possible stimulation of nicotinic as well as muscarinic receptors. Still unclear is the importance of AChE accumulated in the neuropathological structures of AD brains and its possible involvement in the processing of amyloid precursor protein, the protein from which the amyloid deposit in AD plaques originate possibly by false processing. In this context the potential use of centrally acting AChE-I's, also in regard to the progression of the disease, has to be addressed.

SYMPOSIUM ABSTRACTS

## S56 SUBTYPE SELECTIVE MUSCARINIC AGONISTS: POTENTIAL THERAPEUTIC AGENTS FOR ALZHEIMER'S DISEASE. Robert E. Davis, Parke-Davis Pharmaceutical Research, Warner-Lambert Co., Ann Arbor, MI 48106

Postsynaptic muscarinic receptors on cholinceptive neurons in the neocortex and hippocampus are largely intact in Alzheimer's Disease. Muscarinic agonists acting directly at these sites and mimicking the actions of acetylcholine should restore lost cholinergic function and retain efficacy throughout this disease. Unfortunately, the clinical efficacy of muscarinic agonists (i.e. RS 86, arecoline, pilocarpine and oxotremorine) in AD is equivocal. These agents have poor oral bioavailability, short durations of action or unfavorable peripheral parasympathetic profiles that limit their therapeutic utility. None of these 'first generation' agonists were specifically designed as treatments for AD and newer agents that overcome the problems associated with these older muscarinic agonists are needed. CI-979, an oxime analogue of arecoline, overcomes many of the problems associated with these first generation muscarinic agonists. Studies in man and animals suggest that CI-979 is an orally-active, partial- muscarinic agonist with a reasonable duration of action. Unlike most first generation muscarinic agonists (arecoline, oxotremorine, bethanechol) that bind with higher affinity at  $m_2$  than  $m_1$  muscarinic receptor subtypes, CI-979 binds with equal affinity at all muscarinic receptor subtypes. As expected of a non-selective muscarinic agonist, peripheral cholinergic signs are dose limiting after CI-979 treatment in man and non-human primates (i.e. increased perspiration, salivation and GI activity). In animals central cholinomimetic activity appears at doses of CI-979 below those producing overt signs of excessive parasympathetic stimulation. The focus of continued study of CI-979 in man is to determine if efficacy in AD can be demonstrated at doses below those required to elicit unwanted cholinergically-mediated effects. The preclinical and clinical profile of CI-979 may not be optimal. Reducing dose limiting side-effects while retaining central cholinomimetic activity may be required to achieve maximal efficacy with a muscarinic agonist in AD. Based on the distribution of muscarinic receptor subtypes in the central and peripheral nervous system, a  $m_1$  receptor subtype selective agonist should be better tolerated and perhaps have greater efficacy in improving cognitive function than a non-selective agent. We recently identified a novel class of ' $m_1$  selective' partial muscarinic agonists. In rodents these agents improve cognitive function at doses well below those required to simulate GI motility and other signs of peripheral cholinomimetic activity. If this pharmacologic profile can be extended to man, then truly efficacious agents for the palliative treatment of human cognitive dysfunction may be on the horizon.

## S57 CLINICAL ASPECTS OF CHOLINERGIC PHARMACOLOGY: DISCUSSION

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Studies in animal models strongly suggest that pharmacological manipulation of the surviving cholinergic neurons could be efficacious in the symptomatic treatment of Alzheimer disease (AD). In spite of encouraging preclinical findings, clinical applications have often been limited and disappointing indicating our present limitations in testing drugs for dementia in animal models. Among the identifiable problems we may consider the following:

1. It is difficult to model in animals the disease-related, not only age-related, changes in memory observed in AD patients. Reliable transgenic animals are not available yet.
2. Behavioral systems modelling only deficits in acquisition and perception are of limited value as drug models of AD.
3. There are features which distinguish dementia from amnesia which cannot be reproduced in animals (language, praxis, judgement, etc.).
4. Dosages are generally much higher in experimental models than in clinical applications.
5. In vitro models (brain slices, tissue cultures, synaptosomes, brain fractions) do not reflect the complexity of drug-responses in vivo and the interaction with multiple neurotransmitter systems.
6. Putative clinical efficacy and tolerance are difficult to measure under static, non-progressive experimental conditions used to simulate some disease aspects.

The development of more effective models for preclinical screening of potential drug candidates is a necessary step. (Supported by SIU Central Research Committee; and National Institute on Aging Grant #P30 AG08014).

## POSTER ABSTRACTS

P35

## RIGID ANALOGS OF ACETYLCHOLINE (ACh) AS M1 AGONISTS: IMPLICATIONS FOR A RATIONAL TREATMENT STRATEGY IN ALZHEIMER'S DISEASE (AD)

A. Fisher, D. Barak, E. Heldman, D. Gurwitz, R. Haring, R. Brandeis, Z. Pittel, Y. Segall, H. Meshulam, D. Marciano, Z. Samocha, I. Markovitch, E. Shirin, Z. Vogel, and I. Karton.

Israel Inst. Biolog. Res., Ness-Ziona, and The Weizmann Inst., Rehovot, ISRAEL.

M1 agonists, M2 or M3 antagonists were suggested as a treatment strategy in AD. Yet, there is a dearth of selective M1 agonists. Rigid analogs of ACh can be designed for selective actions at muscarinic receptor (mAChR) subtypes. (Cis)-2-methyl-spiro(1,3-oxathiolane-5,3')quinuclidine (AF102B), 2-methyl-spiro(1,3-oxazoline-5,3') quinuclidine (AF125), 2-methyl-spiro(1,3-oxazoline-5,4')-N-methyl-piperidine (AF150) and 2-methyl-spiro(3,1-oxazoline-5,4')-N-methyl-piperidine (AF151) are rigid analog of ACh in which the muscarinic pharmacophore is "frozen" in a conformation ranging from utmost rigidity (AF125), through AF102B, to relatively semi-rigid structures (AF150 & AF151). Whilst AF125 is an M2 > M1 agonist, AF102B, AF150 and AF151 are centrally active M1 agonists. AF102B has a unique profile showing, *inter alia*: only part of the M1 electrophysiology of ACh; unusual binding parameters to mAChRs. AF150 and AF151 are more efficacious agonists than AF102B for M1 AChRs in rat cortex. Unlike AF102B, AF150 and AF151 are full agonists in Chinese hamster ovary (CHO) cells stably transfected with rat and human m1AChR (phosphoinositides hydrolysis and arachidonic acid release), yet partial agonists (25% of carbachol) on these m1AChRs (stimulation of cAMP accumulation). In CHO cells transfected with human m1-m5AChRs and assayed by changes in intracellular  $Ca^{2+}$  levels: AF102B showed m1 full & m3 partial agonistic profile, no effects on m4 and m5AChRs and very weak m2 agonistic effects; AF150 and AF151 showed m1, m3 and m5 full agonistic profile, yet no effect on m2 and antagonistic effect on m4AChRs. These rigid compounds may be utilized as templates to study the various mAChRs pharmacophores using computer-assisted molecular modelling. Moreover, the M1(m1) selectivity of AF102B, AF150, and AF151 may reach beyond the active site of the mAChRs, imposing activation of only distinct G protein subset(s). This might have a clinical significance, inasmuch as in brains from AD patients certain G proteins are altered (Mol. Brain Res. 10:71, 1991). In various animal models for AD all three agonists, and in particular AF102B, exhibited positive effects on mnemonic processes and a wide safety margin. Such agonists, and especially AF102B, can be considered as a rational treatment strategy in AD.

Supported in part by Snow Brand, Japan.

P36

## PRECLINICAL STRATEGIES FOR SYMPTOMATIC TREATMENT OF COGNITIVE DEFICITS SEEN IN ALZHEIMER'S DISEASE: FOCUS ON CHOLINERGIC MECHANISMS. E. B. De Souza.

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Cognitive dysfunction is a prominent symptom seen in aging and in Alzheimer's disease (AD).

The dementia occurring in AD is associated with dysfunction and death of neurons in a variety of cell populations, including cholinergic, monoaminergic, and peptidergic systems which may contribute to the cognitive deficits seen in the disease. A variety of data substantiate a central role for acetylcholine (ACh) in the cognitive dysfunction seen in both aging and AD. Cerebral cortical ACh synthesis and release decline as a function of age in experimental animals and marked deficits in cognitive performance and cerebral cortical cholinergic markers can be induced in experimental animals by lesioning cholinergic cell bodies in the nucleus basalis magnocellularis projecting to the neocortex. These cognitive deficits in animals can be attenuated by muscarinic cholinergic agonists, acetylcholinesterase inhibitors and ACh releasing agents. Furthermore, many of these cholinergic drugs have been proposed and tested as symptomatic therapies for cognitive disorders in man. Limitations of cholinesterase inhibitors include the potential for neuronal overload, while limitations of direct muscarinic agonist therapy include the potential for the distortion of temporal patterns in neuronal transmission and the down-regulation of receptors that is typically associated with chronic agonist administration. More novel preclinical strategies include the development of a class of compounds [linopirdine (DuP 996)] which enhance  $K^{+}$ -stimulated release of a variety of neurotransmitters including ACh, dopamine, and serotonin in brain slices, without effects on basal neurotransmitter release. Furthermore, linopirdine enhances cerebral cortical ACh release *in vivo* in rats and has significant "memory enhancing" effects in a variety of animal models of learning and memory. This mode of action should result in an improvement in deficient neuronal transmission without overload toxicity.

POSTER ABSTRACTS

**P87 ACETYLCHOLINESTERASE AND ITS ASSOCIATION WITH THE HEPARAN SULPHATE GLYCOSAMINOGLYCANS IN CORTICAL AMYLOID DEPOSITS OF ALZHEIMER DISEASE**  
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Previous studies have employed sensitive histochemical techniques to demonstrate acetylcholinesterase (AChE) and butyrylcholinesterase within the pathological lesions of Alzheimer disease. Here we used this technique to show that AChE localized in either frozen or fixed neocortical tissue sections is removed after treatment with various glycosaminoglycans, heparinases or proteases. Heparan sulphate, heparinase lyase type I and to a lesser degree heparin and chondroitin sulphate were effective in solubilizing a large part of the cholinesterase activity. At physiological concentrations, the protease, papain or trypsin readily removed activity but collagenase or pronase were only moderately effective. Peptide protease inhibitors and divalent metals did not exhibit any effect. The specificity of these observations was shown by inhibition of activity with various anticholinesterases including diisofluorophosphate. Our results suggest that AChE is anchored to and may be released from the heparan sulphate glycosaminoglycans previously demonstrated (by immunocytochemical methods and bFGF binding) to be contained in the lesions. We further suggest the localization of cholinesterases is closely associated with the accumulation of the glycosaminoglycans in amyloid containing lesions namely senile plaques and neurofibrillary tangles.

**P88 CEREBROVASCULAR CHOLINERGIC CHANGES IN ALZHEIMER'S DEMENTIA.** N. Zamar, Y. Robitaille, E. Kravitz\* and E. Hamel, Montreal Neurological Institute and \*Montreal General Hospital, McGill University, 3801 University Street, Montréal, Canada H3A 2B4

Alzheimer's dementia (AD) has long been associated with neurotransmitter dysfunctions within the CNS. Recently, however, changes have been reported at the level of peripheral and autonomic functions in AD suggesting that the neurodegenerative process is not restricted to the CNS. In the present study, we have evaluated the parasympathetic cholinergic innervation of cerebral blood vessels in AD patients and elderly controls (C). More specifically, pre- and post-synaptic cholinergic markers were determined in pial blood vessels obtained post-mortem from eight histopathologically proven cases of AD and eight non-demented or neurologically normal elderly subjects (C), matched for age (AD:  $75 \pm 4$  y; C:  $78 \pm 4$  y), post-mortem delay (AD:  $27 \pm 8$  h; C:  $27 \pm 6$  h) and, whenever possible sex (M/F: 2/6 (AD) and 4/4 (C)). The activity of the acetylcholine synthesizing enzyme, choline acetyltransferase (ChAT), was measured as an index of cerebrovascular cholinergic innervation. Muscarinic binding site parameters (affinity ( $K_D$ ) and capacity ( $B_{max}$ )) were determined in cerebrovascular membrane preparations by saturation studies with the non-selective radioligand [ $^3$ H]-N-methylscopolamine ([ $^3$ H]NMS). ChAT activity was slightly lower in AD as compared to C ( $2.1 \pm 0.7$  and  $3.0 \pm 0.9$  nmol/mg protein/hr, respectively). However, a large variability was observed within each group and values were not significantly different. The  $B_{max}$  for [ $^3$ H]-NMS binding in cerebrovascular membranes from AD patients was significantly decreased (43%,  $p < 0.05$ ) as compared to control values ( $50.2 \pm 7$  and  $88.7 \pm 18$  fmol/mg protein, respectively), but the affinity was unaltered ( $K_D$  of  $147 \pm 43$  and  $195 \pm 49$  pM, respectively). When [ $^3$ H]-NMS binding was performed in the presence of 75 nM pirenzepine in order to block all  $M_1$  sites (and a fraction of putative cerebrovascular  $M_4$  sites), remaining sites were still significantly decreased (46%,  $p < 0.05$ ) in AD as compared to C ( $24.2 \pm 3.2$  and  $44.8 \pm 8.3$  fmol/mg protein, respectively). These results clearly show that cerebrovascular muscarinic receptors, not restricted to the  $M_1$  subtype, are severely decreased in AD even though a clear deficit in cholinergic perivascular innervation could not be evidenced. Such alterations could result in abnormal brain perfusion and blood flow regulation. Supported by the MRC of Canada.

POSTER ABSTRACTS

## P89 PHOSPHATIDYL CHOLINE FOR OLFACTORY PROBLEMS

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Since acetylcholine appears essential in the neurotransmission of olfaction, we hypothesized that acetylcholine deficiency may cause olfactory dysfunction. Phosphatidylcholine has been shown to increase acetylcholine in the brain therefore we tested our hypothesis by giving oral doses of PhosChol, 9 gm/day, to each of ten anosmic or hyposmic patients in an open label pilot study. After three months of treatment, four of the ten said they had improved. Therefore, we proceeded with a second study: a double blind trial of the drug against placebo with 20 anosmic or hyposmic patients. However, after three months of treatment, neither experimental subjects nor controls showed measureable positive response or significant change. Further, any subjective changes did not correlate with measured changes, either among experimental or control subjects. The conflicting results between our pilot study and subsequent double blind experiment may be accounted for in various ways: too few subjects can bias results toward false negatives. Patients who dropped out of the experiment because they disliked the taste (smell) of their oral doses may have skewed the results negatively, a positive response to treatment making them more sensitive to a taste they disliked. The placebo may have unknown effects of preventing further deterioration. Or the dosage of PhosChol may have been too small; a larger dosage may be found to yield positive results. Patients' noncompliance is a possibility since blood levels of acetylcholine were not measured. And finally, olfactory tests other than those we used might have revealed a positive response.

P90 DOES MYASTHENIA GRAVIS AFFECT HEARING? Heilbronn E\* and Plinkert P. \*Inst of Neurochemistry and Neurotoxicology, Stockholm University, S-106 91 Stockholm, Sweden and <sup>0</sup>HNO, Univ. Tübingen, Germany.

Myasthenia gravis is an autoimmune disease. In a large number of cases circulating antibodies directed against nicotinic acetylcholine receptors (nAChR) of the peripheral skeletal muscle type exist and block or reduce the number of functional nAChR at the neuromuscular junction of humans and experimental animals. In connection with the identification of the neuroreceptors present at outer haircells (OHC) of the inner ear of mammals, we have recently demonstrated the existence there of a nAChR. According to our pharmacological/biochemical analysis this nAChR closely resembles skeletal muscle nAChR. We have further found that nAChR antibodies present in sera of MG patients bind to OHC (blind tests, OHC in culture or *in situ*, immunocytochemistry). Thus, complaints from MG patients about hearing disturbances may have a real functional background. Objective and highly sensitive hearing studies of experimental animals and patients combined with antibody analysis are in progress.

1. P.K. Plinkert, H.P. Zenner and E. Heilbronn

A nicotinic acetylcholine receptor-like  $\mathcal{L}$ -bungarotoxin-binding site in outer hair cells. Hearing Research 53 (1991) 123-130.

## SESSION 14: CLINICAL ASPECTS OF CHOLINERGIC PHARMACOLOGY

### POSTER ABSTRACTS

#### P91 CHOLINERGIC MECHANISMS IN SCHIZOPHRENIA : RELATIONSHIP TO POSITIVE AND NEGATIVE SYMPTOMS Rajiv Tandon, John B. DeQuardo, James E. Shipley, JoAnn Goodson, John E. Greden

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Although the cholinergic (ACh) system generally receives little attention in schizophrenia, considerable evidence suggests that ACh mechanisms may play a significant role in schizophrenic pathophysiology. Over the past five years, we have been conducting a series of pharmacological, neuroendocrine, and polysomnographic studies designed to evaluate the role of the ACh system in schizophrenia. Based on the association of decreasing cholinergic activity with positive symptoms and the association of increased cholinergic activity with negative symptoms (*Tandon and Greden, Archives of General Psychiatry, 1989, 46 : 745-753*), we proposed the following model of dopaminergic (DA)/cholinergic (ACh) interactions in schizophrenia : (i) disruption of DA/ACh balance is of critical importance in schizophrenic pathophysiology; (ii) ACh activity increases in a "homeostatic" attempt to maintain this balance in the face of increasing DA activity that occurs in the psychotic phase of the illness; (iii) this increased ACh activity exerts a damping effect on the production of positive symptoms associated with DA hyperactivity; and (iv) this compensatory increase in ACh activity is, in turn, accompanied by an increase in negative symptoms. We have been conducting a series of pharmacologic and sleep studies to test predictions of this model. Schizophrenic patients show impaired sleep continuity, shortened REM latency, and normal REM density in comparison to normal controls; these findings are consistent with concomitant increases in DA and ACh activity in schizophrenia. Anticholinergics increase positive symptoms and decrease negative symptoms in schizophrenic patients. We have recently studied the effects of anticholinergic modulation on positive/negative symptoms and polysomnographic measures in drug-free schizophrenic patients, employing a double-blind, placebo-crossover design. Our findings further implicate the cholinergic system in schizophrenic pathophysiology and suggest a role for dopaminergic/ACh interactions at least in the production of sleep abnormalities and expression of positive and negative symptoms. These data indicate the need for systematic trials of cholinergic and anticholinergic agents in the treatment of positive and negative symptoms of schizophrenia, respectively.

## CLOSING LECTURE

### OVERVIEW AND FUTURE DIRECTIONS OF CNS CHOLINERGIC MECHANISMS

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In 1983 I wrote for TIPS an inventory of our knowledge on the 50th anniversary of ACh discovery in the brain. This is an occasion for bringing the inventory up-to-date. At that time, the map of the cholinergic pathways had been just drawn, but still contained many question marks, and the degeneration of the forebrain cholinergic neurons was considered the main pathogenetic component of Alzheimer's dementia. In the last 10 years, the rostral projections of the brain stem cholinergic neurons have been traced, removing most of the question marks, and the modulatory influence of the forebrain and brain stem cholinergic systems on many neuronal systems has been shown. Several peptides are colocalized with ChAT immunoreactivity but, if they are coreleased with ACh, understanding their pre and postsynaptic functions should be a future research direction. While the presynaptic machinery regulating ACh synthesis and release has been reasonably well established, the discovery of 5 muscarinic receptors is a challenge for the pharmacologists and medicinal chemists. In order to understand their functions new specific agonists and antagonists are needed. The cholinergic neurons age rapidly but may recover with NGF, and serine phospholipid treatments. Investigations on cholinergic aging and trophic factors are relevant to neurobiology of aging and may result in useful therapeutic approaches. Finally, the pivotal role of cholinergic neurons in cognitive functions has been amply demonstrated but it remains to be understood why so little cognitive improvement results from activation of cholinergic receptors in normal and pathological conditions.

# **FIRST AUTHOR INDEX**

<u>NAME</u>	<u>ABSTRACT</u>	<u>PAGE</u>
Abdulla, F.A. . . . .	P70	67
Abdulla, F.A. . . . .	P71	68
Aquilonius, S.-M . . . . .	S5	14
Asermely, K.E. . . . .	P48	48
Askalan, R. . . . .	P26	32
Aubert, I. . . . .	P82	75
Bazan, N.G. . . . .	P68	64
Béjanin, S. . . . .	P27	33
Blusztajn, J.K. . . . .	P35	37
Butcher, L.L. . . . .	S2	10
Cadman, E.D. . . . .	P12	21
Calaminici, M. . . . .	P69	67
Caumonti, F. . . . .	P80	74
Caulfield, M.P. . . . .	S35	55
Chatrenet, B. . . . .	P15	22
Chédotal, A. . . . .	P3	13
Chiappinelli, V.A. . . . .	P55	56
Clarke, P.B.S. . . . .	S6	14
Cohen, R.I. . . . .	P66	63
Consolo, S. . . . .	S23	39
Constanti, A. . . . .	P56	56
Cuello, A.C. . . . .	S32	50
Davis, R.E. . . . .	S56	81
Delot, E. . . . .	P46	47
De Souza, E.B. . . . .	P86	82
Di Giulio, A.M. . . . .	P53	52
Dunant, Y. . . . .	S28	46
Dunnnett, S.B. . . . .	S46	69
Eder-Colli, L. . . . .	P49	48
Ehret-Sabatier, L. . . . .	P16	23
El-Bizri, H. . . . .	P13	21
El-Bizri, H. . . . .	P14	22
Enz, A. . . . .	S55	80
Ferguson, S.S.G. . . . .	P23	31
Fibiger, H.C. . . . .	S47	70
Figueiredo, B.C. . . . .	P50	51
Fisher, A. . . . .	P85	82
Fisone, G. . . . .	P42	43
Flentge, F. . . . .	P36	40
Franco-Obrégon, A. . . . .	P61	61
Gage, F.H. . . . .	S29	49
Gauthier, S. . . . .	S54	80
Garofalo, L. . . . .	P51	51
Giacobini, E. . . . .	S57	81
Gillin, J.C. . . . .	S48	76
Ginsberg, D. . . . .	P18	26
Giovannini, M.G. . . . .	P43	43
Giradi, E. . . . .	P74	71
Gisiger, V. . . . .	P19	27
Gorio, A. . . . .	P54	53
Grassi, F. . . . .	P62	61



NAMEABSTRACTPAGE

Gyls, K.H. . . . .	P22	30
Hanin, I. . . . .	S52	79
Hanisch, U.-K. . . . .	P44	44
Hefti, F. . . . .	S31	50
Heilbronn, E. . . . .	S13	24
Heilbronn, E. . . . .	P90	84
Hirsch, A.R. . . . .	P89	84
Hobson, J.A. . . . .	S49	76
Hokin, L.E. . . . .	S37	59
Israel, M. . . . .	S26	45
Iversen, L.L. . . . .	S53	79
Jackson, D.A. . . . .	P39	41
Janson, V.E. . . . .	P33	36
Jones, B.E. . . . .	S1	10
Jones, B.E. . . . .	S51	77
Jones, S.V.P. . . . .	P8	19
Joze, R.S. . . . .	S38	59
Kalaria, R.N. . . . .	P87	83
Karcmar, G. . . . .	S33	54
Kudo, Y. . . . .	S39	60
Krnjevic, K. . . . .	S34	54
Ladinsky, H. . . . .	P7	17
Ladinsky, H. . . . .	S9	18
Lai, J. . . . .	P9	19
Liberini, P. . . . .	P52	52
Linville, D.G. . . . .	P6	16
Lipton, P. . . . .	P72	68
Liu, C.-G. . . . .	P63	62
Liu, C.-G. . . . .	P64	62
Liu, C.-G. . . . .	P65	63
Löffelholz, K. . . . .	S22	38
Massoulié, J. . . . .	S14	24
McBride, W.G. . . . .	P45	44
McCormick, D.A. . . . .	S36	55
McKinney, M. . . . .	S40	60
Messier, C. . . . .	P78	73
Nabeshima, T. . . . .	S45	69
Nabeshima, T. . . . .	P75	72
Norel, X. . . . .	P20	27
Ohue, T. . . . .	P37	40
Ohue, T. . . . .	P38	41
O'Regan, S. . . . .	P29	34
Palacios, J.M. . . . .	S8	15
Paquet, M. . . . .	P11	20
Parsons, S.M. . . . .	S19	29
Patrick, J. . . . .	S10	18
Pemberton, K.E. . . . .	P58	57
Pepou, G. . . . .		86
Petrenko, A.G. . . . .	S27	46
Pilar, G. . . . .	P57	57
Plomp, J.J. . . . .	P47	47
Prado, M.A.M. . . . .	P24	31

NAMEABSTRACTPAGE

Price, D.L. . . . .	S3	11
Prior, C.P. . . . .	P59	58
Quirion, R. . . . .	S7	15
Rasmussen, D.D. . . . .	S42	65
Rodriguez de Lores Arnaiz, G. . . . .	S25	45
Roeske, W.R. . . . .	P10	20
Rylett, R.J. . . . .	S17	28
Salem, N. . . . .	P30	34
Salvaterra, P. . . . .	S18	28
Sastry, B.V.R. . . . .	P34	36
Sastry, B.V.R. . . . .	P67	64
Schmidt, B.M. . . . .	P32	35
Schneider, P.G. . . . .	P77	73
Scremin, O.U. . . . .	S21	38
Scremin, O.U. . . . .	P40	42
Samba, K. . . . .	P83	78
Shiraki, T. . . . .	P41	42
Sillito, A.M. . . . .	S44	66
Silman, I. . . . .	P17	26
Smith, L.K. . . . .	P31	35
Soreq, H. . . . .	S15	25
Steriade, M. . . . .	S50	77
Strauss, W.L. . . . .	P28	33
Tamer, E. . . . .	P76	72
Tandon, A. . . . .	P25	32
Tandon, R. . . . .	P91	85
Tian, L. . . . .	P60	58
Tucek, S. . . . .	S24	39
Turner, J.J. . . . .	P79	74
Umbriaco, D. . . . .	P1	12
Vannucchi, M.G. . . . .	P5	16
Wainer, B.H. . . . .	S4	11
Welner, S.A. . . . .	P81	75
Whittaker, V.P. . . . .	S16	25
Whittaker, V.P. . . . .	P21	30
Widén, L. . . . .	P4	13
Williams, L.R. . . . .	S30	49
Woody, C.D. . . . .	S43	65
Yeomans, J.S. . . . .	P73	71
Yeomans, J.S. . . . .	P84	78
Zaborszky, L. . . . .	P2	12
Zamar, N. . . . .	P88	83

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